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(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
25 May 2001 (25.05.2001)

PCT

(10) International Publication Number
WO 01/36640 A2

(51) International Patent Classification[7]: C12N 15/12, C07K 14/50, C12N 5/10, C07K 16/22, A61K 38/18

(21) International Application Number: PCT/US00/31745

(22) International Filing Date:
16 November 2000 (16.11.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/166,540 18 November 1999 (18.11.1999) US
60/203,633 11 May 2000 (11.05.2000) US

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(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *Without international search report and to be republished upon receipt of that report.*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: HUMAN FGF-21 GENE AND GENE EXPRESSION PRODUCTS

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human FGF-21      MDSDETGFEHSGLWVSVLAGLLLGACQAHPIPDSSPLLQFG-GQV-RQRYLYTD  52
                             ** * * * *   * **  * *  * ***
mouse FGF-15      MARKWNGRAVARALVLATLWLAVS-GRPLAQ-QSQSVSDEDPLFLYGWGKITRLQYLYSA  58

                  DAQQTEAHLEIREDGTVGGAADQSPESLLQLKALKPGVIQILGVKTSRFLCQRPDGALYG 112
                        * ** ** *   **   **  *   * * *  * **  ** **
                  GPYVSNCFLRIRSDGSVDCEEDQNERNLLEFRAVALKTIAIKDVSSVRYLCMSADGKIYG 118

                  SLHFDPEACSFRELLLEDGYNVYQSEAHGLPLHLPGNKSPHRDPAPRGPARFLPLPGLPP 172
                       * * ***    *** * *  * *      * *      * * *
                  LIRYSEEDCTFREEMDCLGYNQYRSMKHHLHIIFIQAK-PREQLQDQKPSNFIPVFHRSF 177

                  ALPEPPGILAPQ--PPDVGSSDPLSMVGPSQG--RSPSYAS
                               *   * ** **       ***
                  FETGDQLRSKMFSLPLESDSMDPFRMVEDVDHLVKSPSFQK
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(57) Abstract: This invention relates to human fibroblast growth factor (hFGF-21), and to variants thereof and to polynucleotides encoding FGF-21. The invention also relates to diagnostic and therapeutic agents related to the polynucleotides and proteins, including probes and antibodies, and to methods of treating liver disease such as cirrhosis and cancer, methods of treating conditions related to thymic function, and methods of treating conditions of the testis. The invention also relates to mouse fibroblast growth factor (mFGF-21), and to variants thereof and polynucleotides encoding mFGF-21.

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HUMAN FGF-21 GENE AND GENE EXPRESSION PRODUCTS

TECHNICAL FIELD

The present invention relates to nucleic acid sequences encoding a member of the fibroblast growth factor (FGF) family, and to polypeptides encoded by the nucleic acid sequence.

BACKGROUND OF THE INVENTION

The prototypic fibroblast growth factors (FGFs), FGF-1 and FGF-2, were originally isolated from brain and pituitary as mitogens for fibroblasts. However, FGF-1 and FGF-2 are widely expressed in developing and adult tissues, and are polypeptides with multiple biological activities including angiogenesis, mitogenesis, cellular differentiation and repair of tissue injury (Baird, A. et al., *Cancer Cells 3*:239-243 (1991); Burgess, W.H. et al., *Annu. Rev. Biochem. 58*:575-606 (1989). According to the published literature, the FGF family now consists of at least nineteen members, FGF-1 to FGF-19. FGF-3 was identified to be a common target for activation by the mouse mammary tumor virus (Dickson et al., *Ann. N.Y. Acad. Sci. 638*:18-26 (1991); FGF-4 to FGF-6 were identified as oncogene products (Yoshida et al., *Ann. NY Acad. Sci. 638*:27-37 (1991); Goldfarb et al., *Ann. NY Acad. Sci 638*:38-52 (1991); Coulier et al., *Ann. NY Acad. Sci. 638*:53-61 (1991)). FGF-10 was identified from rat lung by homology-based polymerase chain reaction (PCR) (Yamasaki et al., *J. Biol. Chem. 271*:15918-15921 (1996)). FGF-11 to FGF-14 (FGF homologous factors (FHFs) 1 to 4) were identified from human retina by a combination of random cDNA sequencing, data base searches and homology-based PCR (Smallwood et al., *Proc. Natl. Acad. Sci. USA 93*:9850-9857 (1996)). FGF-15 was identified as a downstream target of a chimeric homeodomain oncoprotein (McWhirter et al., *Development 124*:3221-3232 (1997)). FGF-16, FGF-17, and FGF-18 were identified from rat heart and embryos by homology-based PCR, respectively (Miyake et al., *Biochem. Biophys. Res. Commun. 243*:148-152 (1998); Hoshikawa et al., *Biochem. Biophys. Res. Commun. 244*:187-191 (1998); Ohbayashi et al., *J. Biol. Chem. 273*:18161-18164 (1998)). Recently, FGF-19

was identified from human fetal brain by data base search (Nishimura et al., *Biochim. Biophys. Acta 1444*:148-151 (1999)). They have a conserved ~120-amino acid residue core with ~30 to 60% amino acid identity. These FGFs also appear to play important roles in both developing and adult tissues. Thus, there is a need in the art for additional FGF molecules having functions and activities that differ from the known FGFs and for FGF molecules specifically expressed in tissues implicated in human disease.

SUMMARY OF THE INVENTION

The present invention provides a composition comprising an isolated polynucleotide selected from the group consisting of:

(a) a polynucleotide comprising at least eight contiguous nucleotides of SEQ ID NO:1 or 3;

(b) a polynucleotide having at least 80% homology to the polynucleotide of (a); and

(c) a polynucleotide encoding a protein expressed by a polynucleotide having the sequence of SEQ ID NO:1 or 3.

The invention further provides for the use of the isolated polynucleotides or fragments thereof as diagnostic probes or as primers.

The present invention also provides a composition comprising a polypeptide, wherein said polypeptide is selected from the group consisting of:

(a) a polypeptide comprising at least 6 contiguous amino acids encoded by SEQ ID NO:1 or 3;

(b) a polypeptide encoded by a polynucleotide comprising SEQ ID NO:1 or 3; and

(c) a variant of the polypeptide of SEQ ID NO:2 or 4.

In certain preferred embodiments of the invention, the polynucleotide is operably linked to an expression control sequence. The invention further provides a host cell, including bacterial, yeast, insect and mammalian cells, transformed with the polynucleotide sequence. The invention also provides full-length cDNA and full-length polynucleotides corresponding to SEQ ID NO:1 or 3.

Protein and polypeptide compositions of the invention may further comprise a pharmaceutically acceptable carrier. Compositions comprising an antibody that specifically reacts with such protein or polypeptide are also provided by the present invention.

The invention also provides for the production of large amounts of otherwise minor cell populations of cells to be used for generation of cDNA libraries for the isolation of rare molecules expressed in the precursors cells or progeny; cells produced by treatment may directly express growth factors or other molecules, and conditioned media is screened in assays for novel activities.

The invention further provides for the isolation, self-renewal and survival of mammalian stem cells and the differentiation of their progeny.

The invention also provides for compositions and methods of preventing or slowing the degeneration of or increasing the numbers of hepatic cells, in disease states including but not limited to, cirrhosis of the liver, hepatitis, and post-surgical and post-injury tissue regeneration; of preventing or slowing degeneration of or increasing the numbers of cells in the testes in disease states such as infertility and impotence, and of preventing or slowing degeneration of or increasing the numbers of cells of the thymus in disorders of the thymus and immune system.

The invention also provides for compositions and methods for identifying inhibitors of FGF-21 function, useful in disease states such as liver and testicular cancers, or leukemias, lymphomas or other cancers, and proliferative or differentiation disorders of cells derived from the thymus.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. Amino acid sequence comparison of human FGF-21 with mouse FGF-15. Asterisks indicate identical amino acid residues of the sequences.

Figure 2. Amino acid sequence comparison of human FGF-21 and human FGF-19. Asterisks indicate identical amino acid residues of the sequences.

Figure 3. Expression of FGF-21 in mouse tissues.

Figure 4. DNA sequence (SEQ ID NO:1) and amino acid sequence (SEQ ID NO:2) of mouse FGF-21.

Figure 5. DNA sequence (SEQ ID NO:3) and amino acid sequence (SEQ ID NO:4) of human FGF-21.

Figure 6. Alignment of the amino acid sequences of human (SEQ ID NO:4) and mouse (SEQ ID NO:2) FGF-21.

Figure 7. Figure 7 provides codon usage for yeast. The first field of information on each line of the table contains a three-letter code for an amino acid. The second field contains an unambiguous codon for that amino acid. The third field lists the number of occurrences of that codon in the genes from which the table is compiled. The fourth field lists the expected number of occurrences of that codon per 1,000 codons in genes whose codon usage is identical to that compiled in the codon frequency table. The last field contains the fraction of occurrences of the codon in its synonymous codon family.

Figure 8. Figure 8 provides codon usage for Drosophila.

Figure 9. Figure 9 provides codon usage for E. coli.

DETAILED DESCRIPTION OF THE INVENTION

Because of their potent activities for promoting growth, proliferation, survival and differentiation of a wide variety of cells and tissue types, FGFs continue to be pursued as therapeutic agents for a number of different indications, including wound healing, such as musculo-skeletal conditions, for example, bone fractures, ligament and tissue repair, tendonitis, bursitis, etc.; skin conditions, for example, burns, cuts, lacerations, bed sores, slow healing ulcers, etc.; tissue protection, repair, and the induction of angiogenesis during myocardial infarction and ischemia, in the treatment of neurological conditions, for example, neuro-degenerative disease and stroke, in the treatment of eye disease, including macular degeneration, and the like.

The fibroblast growth factor (FGF) proteins identified to date belong to a family of signaling molecules that regulate growth and differentiation of a variety of cell types. The significance of FGF proteins to human physiology and pathology relates

in part to their key roles in embryogenesis, in blood vessel development and growth, and in bone growth. *In vitro* experiments have demonstrated a role for FGF in regulating cell growth and division of endothelial cells, vascular smooth muscle cells, fibroblasts, and cardiac and skeletal myocytes. Other members of the FGF family and their biological roles are described in Crossley *et al.*, *Development 121*:439-451 (1995); Ohuchi *et al.*, *Development 124*:2235-2244 (1997); Gemel *et al.*, *Genomics 35*:253-257 (1996); and Ghosh *et al.*, *Cell Growth and Differentiation 7*:1425-1434 (1996).

FGF proteins are also significant to human health and disease because of a role in cancer cell growth. For example, FGF-8 was identified as an androgen-induced growth factor in breast and prostate cancer cells. (Tanaka *et al.*, *FEBS Lett. 363*:226-230 (1995) and *P.N.A.S. 89*:8928-8932 (1992)).

The role of FGF in normal development is being elucidated in part through studies of FGF receptors. Wilke, T. et al., *Dev. Dynam. 210*:41-52 (1997) found that FGFR1, FGFR2, and FGFR3 transcripts were localized to specific regions of the head during embryonic development in chickens. The expression pattern correlated with areas affected by human FGFR mutations in Crouzon syndrome, a condition of abnormal intramembranous bone formation. Belluardo, N. et al., *Jour. Comp. Neur. 379*:226-246 (1997) studied localization of FGFR 1, 2, and 3 mRNAs in rat brain, and found cellular specificity in several brain regions. Furthermore, FGFR1 and FGFR2 mRNAs were expressed in astroglial reactive cells after brain lesion, supporting a role of certain FGF's in brain disease and injury. Ozawa, K. et al., *Mol. Brain Res. 41*:279-288 (1996) reported that FGF1 and FGF-5 expression increased after birth, whereas FGF3, FGF-6, FGF-7, and FGF-8 genes showed higher expression in late embryonic stages than in postnatal stages.

New members of the FGF family are described here, wherein the FGF protein is expressed in a variety of tissues but most abundantly in the liver. A polynucleotide encoding the mouse FGF of the invention has the sequence as shown in SEQ ID NO:1. A polynucleotide encoding the human FGF of the invention has the sequence as shown in SEQ ID NO:3. The mouse polynucleotide was identified as encoding a member of the FGF family by the conserved regions throughout the amino

acid sequence and by the regions of homology shared by the polynucleotide and genes encoding known FGF proteins.

The inventors believe that FGF-21 is a previously unidentified member of the FGF family. To date, over 19 human FGF proteins have been identified. In most cases, homologous proteins in other mammals, particularly mice and rats, have also been identified. The human proteins vary to different degrees in terms of amino acid sequence, receptor specificity, tissue expression patterns, and biological activity.

The present FGF-21 differs in sequence from all the FGF proteins described to date in publications. As discussed herein, the knowledge about the roles played by various FGF proteins continues to grow, but is by far incomplete.

The present invention adds to this knowledge by disclosing that the FGF of SEQ ID NO:1 is highly expressed in liver, and human FGF-21 may play a role in development of and recovery from liver disease. Further, FGF-21 is also expressed in testis and thymus, and therefore may play a role in the development or recovery from disorders of testicular function or function of cells derived from the thymus. The invention therefore is based upon the identification, isolation and sequencing of a new fibroblast growth factor (FGF-21).

Isolation and Analysis of Mouse cDNA encoding FGF-21 According to the invention, DNA encoding a novel mouse FGF has been identified. The nucleotide sequence of the entire coding region was determined by adaptor-ligation mediated polymerase chain reaction using mouse embryo cDNA as a template. The nucleotide sequence of the coding region allowed for the elucidation of the complete amino acid sequence of the mouse FGF (210 amino acids) (Figure 4). This protein is tentatively named FGF-21.

Isolation and Analysis of Human cDNA Encoding FGF-21 A human gene encoding FGF-21 was located in the 5′ flanking region of a putative human α-fucosyltransferase gene. The cDNA encoding the entire coding region of human FGF-21 was amplified from fetal brain cDNA by PCR using FGF-specific primers as follows: sense primer: 5′ agccattgatggactcggac 3′ (SEQ ID NO:5); antisense primer: 5′ tggcttcaggaagcgtagct 3′ (SEQ ID NO:6).

Expression of FGF-21 mRNA in Adult Mouse Tissues The expression of FGF-21 mRNA was examined in adult mouse major tissues including brain, heart, lung, liver, kidney, spleen, lung, thymus, testis, muscle, skin, and small intestine by polymerase chain reaction. FGF-21 mRNA expression was detected at high levels in the liver (Figure 3). Expression was also seen in testis and thymus. To confirm the expression of FGF-21 mRNA in mouse tissues, mouse tissue $(A)^+$ RNA was examined by Northern blotting analysis using a ^{32}P-labeled rat FGF-21 cDNA probe. The results confirmed a high level of expression in mouse liver. Expression was also seen in thymus; larger transcripts were seen in testis tissue.

Reference to FGF-21 herein is intended to be construed to include growth factors of any origin which are substantially homologous to and which are biologically equivalent to the FGF-21 characterized and described herein. Such substantially homologous growth factors may be native to any tissue or species and, similarly, biological activity can be characterized in any of a number of biological assay systems.

The term "biologically equivalent" is intended to mean that the compositions of the present invention are capable of demonstrating some or all of the same growth properties in a similar fashion, not necessarily to the same degree as the FGF-21 isolated as described herein or recombinantly produced human FGF-21 of the invention.

By "substantially homologous" it is meant that the degree of homology of human FGF-21 to FGF-21 from any species is greater than that between FGF-21 and any previously reported member of the FGF family.

Sequence identity or percent identity is intended to mean the percentage of same residues between two sequences, referenced to human FGF when determining percent identity with non-human FGF-21, referenced to FGF-21 when determining percent identity with non-FGF-21 growth factors, when the two sequences are aligned using the Clustal method (Higgins et al, *Cabios 8*:189-191, 1992) of multiple sequence alignment in the Lasergene biocomputing software (DNASTAR, INC, Madison, WI). In this method, multiple alignments are carried out in a progressive manner, in which

larger and larger alignment groups are assembled using similarity scores calculated from a series of pairwise alignments. Optimal sequence alignments are obtained by finding the maximum alignment score, which is the average of all scores between the separate residues in the alignment, determined from a residue weight table representing the probability of a given amino acid change occurring in two related proteins over a given evolutionary interval. Penalties for opening and lengthening gaps in the alignment contribute to the score. The default parameters used with this program are as follows: gap penalty for multiple alignment=10; gap length penalty for multiple alignment=10; k-tuple value in pairwise alignment=1; gap penalty in pairwise alignment=3; window value in pairwise alignment=5; diagonals saved in pairwise alignment=5. The residue weight table used for the alignment program is PAM250 (Dayhoff *et al.*, in Atlas of Protein Sequence and Structure, Dayhoff, Ed., NDRF, Washington, Vol. 5, suppl. 3, p. 345, 1978).

Percent conservation is calculated from the above alignment by adding the percentage of identical residues to the percentage of positions at which the two residues represent a conservative substitution (defined as having a log odds value of greater than or equal to 0.3 in the PAM250 residue weight table). Conservation is referenced to human FGF-21 when determining percent conservation with non-human FGF-21, and referenced to FGF-21 when determining percent conservation with non-FGF-21 growth factors. Conservative amino acid changes satisfying this requirement are: R-K; E-D, Y-F, L-M; V-I, Q-H.

The invention provides FGF-21 proteins or variants thereof having one or more polymers covalently attached to one or more reactive amino acid side chains. By way of example, not limitation, such polymers include polyethylene glycol (PEG), which can be attached to one or more free cysteine sulfhydryl residues, thereby blocking the formation of disulfide bonds and aggregation when the protein is exposed to oxidizing conditions. In addition, pegylation of FGF-21 proteins and/or muteins is expected to provide such improved properties as increased half-life, solubility, and protease resistance. FGF-21 proteins and/or muteins may alternatively be modified by the covalent addition of polymers to free amino groups such as the lysine epsilon or the

N-terminal amino group. Preferred cysteines and lysines for covalent modification will be those not involved in receptor or heparin binding or in proper protein folding. For example, cys 27 and cys 104 may be modified. It will be apparent to one skilled in the art that the methods for assaying FGF-21 biochemical and/or biological activity may be employed in order to determine if modification of a particular amino acid residue affects the activity of the protein as desired.

It may be advantageous to improve the stability of FGF-21 by modifying one or more protease cleavage sites. Thus, the present invention provides FGF-21 variants in which one or more protease cleavage site has been altered by, for example, substitution of one or more amino acids at the cleavage site in order to create an FGF-21 variant with improved stability. Such improved protein stability may be beneficial during protein production and/or therapeutic use. A preferred site is a monobasic site within two residues of a proline, such as near residue 160 of SEQ ID NO:4.

Suitable protease cleavage sites for modification are well known in the art and likely will vary depending on the particular application contemplated. For example, typical substitutions would include replacement of lysines or arginines with other amino acids such as alanine. The loss of activity, such as receptor binding or heparin binding, can be tested for as described herein.

FGF-21 can also include hybrid and modified forms of FGF-21 including fusion proteins and FGF-21 fragments and hybrid and modified forms in which certain amino acids have been deleted or replaced and modifications such as where one or more amino acids have been changed to a modified amino acid or unusual amino acid and modifications such as glycosylations so long as the hybrid or modified form retains the biological activity of FGF-21. Fusion proteins can consist of the FGF-21 of the invention or fragment thereof and a signal sequence of a heterologous protein to promote secretion of the protein product.

Fusion proteins comprising FGF-21 or a biologically active or antigenic fragment thereof can be produced using methods known in the art. Such fusion proteins can be used therapeutically or can be produced in order to simplify the isolation and purification procedures. Histidine residues can be incorporated to allow immobilized

metal affinity chromatography purification. Residues EQKLISEEDL contain the antigenic determinant recognized by the myc monoclonal antibody and can be incorporated to allow myc monoclonal antibody-based affinity purification. A thrombin cleavage site can be incorporated to allow cleavage of the molecule at a chosen site; a preferred thrombin cleavage site consists of residues LVPRG. Purification of the molecule can be facilitated by incorporating a sequence, such as residues SAWRHPQFGG, which binds to paramagnetic streptavidin beads. Such embodiments are described in WO 97/25345, which is incorporated by reference.

The invention further includes chimeric molecules between FGF-21 and keratinocyte growth factor (KGF) (Reich-Slotky, R. et al., *J. Biol. Chem. 270*:29813-29818 (1995)). The chimeric molecule can contain specific regions or fragments of one or both of the FGF-21 and KGF molecules, such as the FGF-21 fragments described below.

The invention also includes fragments of FGF-21. Preferred fragments of SEQ ID NO:4 and 2, respectively, include: amino acids from about 1 to about 209 (210 for SEQ ID NO:2); amino acids from about 2 to about 209 (210 for SEQ ID NO:2); amino acids from about 1 to about 177; amino acids from about 40 to about 209 for SEQ ID NO:2 and amino acids from about 40 to about 177. Such fragments can be prepared from the proteins by standard biochemical methods, or by expressing a polynucleotide encoding the fragment.

FGF-21, or a fragment thereof, can be produced as a fusion protein comprising human serum albumin (HSA) or a portion thereof. Such fusion constructs are suitable for enhancing expression of the FGF-21, or fragment thereof, in an eukaryotic host cell. Exemplary HSA portions include the N-terminal polypeptide (amino acids 1-369, 1-419, and intermediate lengths starting with amino acid 1), as disclosed in U.S. Patent No. 5,766,883, and publication WO 97/24445, incorporated by reference herein. Other chimeric polypeptides can include a HSA protein with FGF-21, or fragments thereof, attached to each of the C-terminal and N-terminal ends of the HSA. Such HSA constructs are disclosed in U.S. Patent No. 5,876,969, incorporated by reference herein.

Also included with the scope of the invention are FGF-21 molecules that differ from native FGF-21 by virtue of changes in biologically active sites.

Growth factors are thought to act at specific receptors. According to the invention, FGF-21 and as yet unknown members of this family of growth factors act through specific receptors having distinct distributions as has been shown for other growth factor families.

A preferred hFGF-21 of the present invention has been identified. Also preferred is hFGF-21 prepared by recombinant DNA technology. Included within the scope of the invention are polynucleotides, including DNA and RNA, with 80% homology to SEQ ID NO:1 or SEQ ID NO:3; preferably at least 85% homology, more preferably at least 90% homology, most preferably 95% homology. Polynucleotides with 96%, 97%, 98%, and 99% homology to SEQ ID NO:1 or 3 are also included. Percent homology is calculated using methods known in the art. A non-limiting example of such a method is the Smith-Waterman homology search algorithm as implemented in MPSRCH program (Oxford Molecular), using an affine gap search with a gap open penalty of 12 and a gap extension penalty of 1.

FGF-21 can also include hybrid and modified forms of FGF-21 including fusion proteins and FGF-21 fragments and hybrid and modified forms in which certain amino acids have been deleted or replaced and modifications such as where one or more amino acids have been changed to a modified amino acid or unusual amino acid and modifications such as glycosylations so long as the hybrid or modified form retains the biological activity of FGF-21. By retaining the biological activity, it is meant that the ability of FGF-21 to promote the growth, survival or differentiation of responsive cells is preserved, although not necessarily at the same level of potency as that of the FGF-21 isolated as described herein or that of the recombinantly produced FGF-21.

Also included within the meaning of substantially homologous is any FGF-21 which may be isolated by virtue of cross-reactivity with antibodies to the FGF-21 described herein or whose encoding nucleotide sequences including genomic DNA, mRNA or cDNA may be isolated through hybridization with the complementary

sequence of genomic or subgenomic nucleotide sequences or cDNA of the FGF-21 herein or fragments thereof. It will also be appreciated by one skilled in the art that degenerate DNA sequences can encode human FGF-21 and these are also intended to be included within the present invention as are allelic variants of FGF-21.

Recombinant human FGF-21 may be made by expressing the DNA sequences encoding FGF-21 in a suitable transformed host cell. Using methods well known in the art, the DNA encoding FGF-21 may be linked to an expression vector, transformed into a host cell and conditions established that are suitable for expression of FGF-21 by the transformed cell.

The DNA encoding FGF-21 can be engineered to take advantage of preferred codon usage of host cells. Codon usage in *Pseudomonas aeruginosa* is described in, for example, West et al., *Nucleic Acids Res. 11*:9323-9335 (1988). Codon usage in *Saccharomyces cerevisiae* is described in, for example, Lloyd et al., *Nucleic Acids Res. 20*:5289-5295 (1992). Codon preference in Corynebacteria and a comparison with *E. coli* preference is provided in Malubres et al., *Gene 134*:15-24 (1993). Codon usage in *Drosophila melanogaster* is described in, for example, Akashi, *Genetics 136*:927-935 (1994). Codon usage in yeast is also shown in Figure 7, codon usage in Drosophila is shown in Figure 8, and codon usage for *E. coli* is shown in Figure 9.

Any suitable expression vector may be employed to produce recombinant human FGF-21 such as expression vectors for use in insect cells. Baculovirus expression systems can also be employed. A preferable method is expression in insect cells, such as Tr5 or Sf9 cells, using baculovirus vector.

The present invention includes nucleic acid sequences including sequences that encode human FGF-21. Also included within the scope of this invention are sequences that are substantially the same as the nucleic acid sequences encoding FGF-21. Such substantially the same sequences may, for example, be substituted with codons more readily expressed in a given host cell such as E. coli according to well known and standard procedures. Such modified nucleic acid sequences are included within the scope of this invention.

Specific nucleic acid sequences can be modified by those skilled in the art and, thus, all nucleic acid sequences that code for the amino acid sequences of FGF-21 can likewise be so modified. The present invention thus also includes nucleic acid sequence which will hybridize with all such nucleic acid sequences, or complements of the nucleic acid sequences where appropriate, and encode a polypeptide having the cell survival, growth or differentiation activity of FGF-21. The present invention also includes nucleic acid sequences that encode polypeptides that have cell survival promoting activity and that are recognized by antibodies that bind to FGF-21. Preferred methods and epitopes for raising antibodies are described in Example 4.

The present invention also encompasses vectors comprising expression regulatory elements operably linked to any of the nucleic acid sequences included within the scope of the invention. This invention also includes host cells of any variety that have been transformed with vectors comprising expression regulatory elements operably linked to any of the nucleic acid sequences included within the scope of the present invention.

Methods are also provided herein for producing FGF-21. Preparation can be by isolation from conditioned medium from a variety of cell types so long as the cell type produces FGF-21. A second and preferred method involves utilization of recombinant methods by isolating or obtaining a nucleic acid sequence encoding FGF-21, cloning the sequence along with appropriate regulatory sequences into suitable vectors and cell types, and expressing the sequence to produce FGF-21.

Although FGF-21 has been described on the basis of its high expression level in liver, this factor may act on other cell types as well. It is likely that FGF-21 will act on non-liver cells to promote their survival, growth, differentiation state or function. This expectation is based upon the activity of known growth factors. Members of the FGF family act on many cell types of different function and embryologic origin, even when their expression is limited to one or a few tissues.

The inventors herein have identified that FGF-21 is expressed at a higher level in liver. This suggests a role for FGF-21 in, for example, precancerous lesions, hepatoma, cirrhosis, repair, from inflammatory diseases, trauma or other types of injury,

and other diseases of the liver. Further, FGF-21 is also expressed in thymus and testis. This suggests a role for FGF-21 in, for example, infertility, control of testosterone production, cancer of the testis or associated cells, and other disorders of the testis, and in disorders of cells such as immune cells derived from the thymus, for example, autoimmune disorders, leukemias and lymphomas, immune deficiency states, and the like.

The present invention also includes therapeutic or pharmaceutical compositions comprising FGF-21 in an effective amount for treating patients with liver, testis or thymic disease, and a method comprising administering a therapeutically effective amount of FGF-21. These compositions and methods are useful for treating a number of diseases. The compositions and methods herein can also be useful to prevent degeneration and/or promote survival in other non-liver tissues as well, such as promoting angiogenesis, neuronal survival, wound healing, and the like. One skilled in the art can readily use a variety of assays known in the art to determine whether FGF-21 would be useful in promoting survival or functioning in a particular cell type. Promotion of neuronal survival is useful in the treatment of nervous system diseases and conditions, including Parkinson's disease, Alzheimers disease, traumatic injury to nerves, and degenerative disease of the nervous system.

In certain circumstances, it may be desirable to modulate or decrease the amount of FGF-21 expressed. Thus, in another aspect of the present invention, FGF-21 anti-sense oligonucleotides can be made and a method utilized for diminishing the level of expression of FGF-21 by a cell comprising administering one or more FGF-21 anti-sense oligonucleotides. By FGF-21 anti-sense oligonucleotides reference is made to oligonucleotides that have a nucleotide sequence that interacts through base pairing with a specific complementary nucleic acid sequence involved in the expression of FGF-21 such that the expression of FGF-21 is reduced. Preferably, the specific nucleic acid sequence involved in the expression of FGF-21 is a genomic DNA molecule or mRNA molecule that encodes FGF-21. This genomic DNA molecule can comprise regulatory regions of the FGF-21 gene, or the coding sequence for mature FGF-21 protein. The term complementary to a nucleotide sequence in the context of FGF-21

antisense oligonucleotides and methods therefor means sufficiently complementary to such a sequence as to allow hybridization to that sequence in a cell, *i.e.*, under physiological conditions. The FGF-21 antisense oligonucleotides preferably comprise a sequence containing from about 8 to about 100 nucleotides and more preferably the FGF-21 antisense oligonucleotides comprise from about 15 to about 30 nucleotides. The FGF-21 antisense oligonucleotides can also contain a variety of modifications that confer resistance to nucleolytic degradation such as, for example, modified internucleoside linages (Uhlmann and Peyman, *Chemical Reviews 90*:543-548 1990; Schneider and Banner, *Tetrahedron Lett. 31*:335, 1990 which are incorporated by reference), modified nucleic acid bases and/or sugars and the like.

The therapeutic or pharmaceutical compositions of the present invention can be administered by any suitable route known in the art including for example intravenous, subcutaneous, intramuscular, transdermal, intrathecal or intracerebral. Administration can be either rapid as by injection or over a period of time as by slow infusion or administration of slow release formulation.

FGF-21 can also be linked or conjugated with agents that provide desirable pharmaceutical or pharmacodynamic properties. For example, FGF-21 can be coupled to any substance known in the art to promote penetration or transport across the blood-brain barrier such as an antibody to the transferring receptor, and administered by intravenous injection (see, for example, Friden *et al.*, *Science 259*:373-377, 1993 which is incorporated by reference). Furthermore, FGF-21 can be stably linked to a polymer such as polyethylene glycol to obtain desirable properties of solubility, stability, half-life and other pharmaceutically advantageous properties. (See, for example, Davis *et al.*, *Enzyme Eng. 4*:169-73, 1978; Burnham, *Am. J. Hosp. Pharm. 51*:210-218, 1994 which are incorporated by reference.)

The compositions are usually employed in the form of pharmaceutical preparations. Such preparations are made in a manner well known in the pharmaceutical art. One preferred preparation utilizes a vehicle of physiological saline solution, but it is contemplated that other pharmaceutically acceptable carriers such as physiological concentrations of other non-toxic salts, five percent aqueous glucose

solution, sterile water or the like may also be used. It may also be desirable that a suitable buffer be present in the composition. Such solutions can, if desired, be lyophilized and stored in a sterile ampoule ready for reconstitution by the addition of sterile water for ready injection. The primary solvent can be aqueous or alternatively non-aqueous. FGF-21 can also be incorporated into a solid or semi-solid biologically compatible matrix which can be implanted into tissues requiring treatment.

The carrier can also contain other pharmaceutically-acceptable excipients for modifying or maintaining the pH, osmolarity, viscosity, clarity, color, sterility, stability, rate of dissolution, or odor of the formulation. Similarly, the carrier may contain still other pharmaceutically-acceptable excipients for modifying or maintaining release or absorption or penetration across the blood-brain barrier. Such excipients are those substances usually and customarily employed to formulate dosages for parenteral administration in either unit dosage or multi-dose form or for direct infusion into the cerebrospinal fluid by continuous or periodic infusion.

Dose administration can be repeated depending upon the pharmacokinetic parameters of the dosage formulation and the route of administration used.

It is also contemplated that certain formulations containing FGF-21 are to be administered orally. Such formulations are preferably encapsulated and formulated with suitable carriers in solid dosage forms. Some examples of suitable carriers, excipients, and diluents include lactose, dextrose, sucrose, sorbitol, mannitol, starches, gum acacia, calcium phosphate, alginates, calcium silicate, microcrystalline cellulose, polyvinylpyrrolidone, cellulose, gelatin, syrup, methyl cellulose, methyl- and propylhydroxybenzoates, talc, magnesium, stearate, water, mineral oil, and the like. The formulations can additionally include lubricating agents, wetting agents, emulsifying and suspending agents, preserving agents, sweetening agents or flavoring agents. The compositions may be formulated so as to provide rapid, sustained, or delayed release of the active ingredients after administration to the patient by employing procedures well known in the art. The formulations can also contain substances that

diminish proteolytic degradation and promote absorption such as, for example, surface active agents.

Depending on the treatment regimen contemplated, it may be desired to control the rate of release of FGF-21 protein or variant thereof to provide long-term treatment while minimizing the frequency of administration. Such treatment regimens may be desired, for example, where the FGF-21 protein is found to be relatively unstable such that the localized concentration of active protein is at an efficacious level for an insufficient period of time. Thus, for example, for certain diseases, it may not be desired or practical to perform repeated and frequent injections. The major advantages of such sustained release systems include targeted local delivery of drugs at a constant rate, less drug required to treat the disease state, minimization of possible side effects, and enhanced efficacy of treatment. Also, these forms of delivery systems are capable of protecting drugs that are unstable *in vivo* and that would normally require a frequent dosing interval. Under such circumstances, sustained release may be achieved by one of the methods readily available in the art such as the encapsulation of FGF-21 conjugated heparin-Sepharose beads to form heparin-alginate microspheres or the preparation of FGF-21 PLG microspheres.

Heparin-alginate microspheres have been successfully employed for the delivery of Basic Fibroblast Growth Factor to tissue (Lopez et al., *Journal of Pharmacology and Experimental Therapeutics 282*(1):385-390 (1997)). Similarly, Alginate/heparin-Sepharose microspheres and films have been used as drug carriers to control the release of a basic FGF-saponin conjugate in order to control its release in small doses. Addition of heparin to solutions of bFGF prevents losses in activity that accompany changes in pH or elevation in temperature. See, for example, Gospodarowicz et al., *J. Cell. Physiol. 128*:475-484 (1986).

Binding of FGF-21 to heparin may be employed in order to enhance its stability either during *in vivo* expression or administration or *in vitro* during various stages of protein purification. Thus, by the present invention, heparin may be added to a solution of FGF-21 and the activity assayed by the methods disclosed herein.

FGF-21 bound heparin-Sepharose beads may be encapsulated into

calcium alginate microspheres to permit the controlled release of the heparin-stabilized FGF-21 protein. For example, microspheres may be constructed by dropping a mixed solution of sodium alginate with FGF-21 bound heparin-Sepharose beads into a hardening solution of calcium chloride. Spheres are formed instantaneously as the mixture enters the hardening solution. The size of the microsphere may be adjusted by passing the FGF-21 bound heparin-Sepharose beads through a cylinder of reduced cross-sectional area such as through a hypodermic needle.

Encapsulation efficiency may be determined by comparing the amount of encapsulated growth factor with that initially present in solution. For example, the FGF-21 may be stripped from the heparin-Sepharose beads with a solution of 3 M NaCl and functional activity assays may be performed.

The specific dose is calculated according to the approximate body weight or body surface area of the patient or the volume of body space to be occupied. The dose will also be calculated dependent upon the particular route of administration selected. Further refinement of the calculations necessary to determine the appropriate dosage for treatment is routinely made by those of ordinary skill in the art. Such calculations can be made without undue experimentation by one skilled in the art in light of the activity disclosed herein in assay preparations of target cells. Exact dosages are determined in conjunction with standard dose-response studies. It will be understood that the amount of the composition actually administered will be determined by a practitioner, in the light of the relevant circumstances including the condition or conditions to be treated, the choice of composition to be administered, the age, weight, and response of the individual patient, the severity of the patient's symptoms, and the chosen route of administration.

In one embodiment of this invention, FGF-21 may be therapeutically administered by implanting into patients vectors or cells capable of producing a biologically-active form of FGF-21 or a precursor of FGF-21, *i.e.*, a molecule that can be readily converted to a biological-active form of FGF-21 by the body. In one approach cells that secrete FGF-21 may be encapsulated into semipermeable membranes for implantation into a patient. The cells can be cells that normally express

FGF-21 or a precursor thereof or the cells can be transformed to express FGF-21 or a precursor thereof. It is preferred that the cell be of human origin and that the FGF-21 be human FGF-21 when the patient is human. However, the formulations and methods herein can be used for veterinary as well as human applications and the term "patient" as used herein is intended to include human and veterinary patients.

Cells can be grown ex vivo for use in transplantation or engraftment into patients (Muench *et al., Leuk. & Lymph. 16*:1-11, 1994 which is incorporated by reference). In another embodiment of the present invention, FGF-21 is used to promote the ex vivo expansion of a cells for transplantation or engraftment. Current methods have used bioreactor culture systems containing factors such as erythropoietin, colony stimulating factors, stem cell factor, and interleukins to expand hematopoietic progenitor cells for erythrocytes, monocytes, neutrophils, and lymphocytes (Verfaillie, *Stem Cells 12*:466-476, 1994 which is incorporated by reference). These stem cells can be isolated from the marrow of human donors, from human peripheral blood, or from umbilical cord blood cells. The expanded blood cells are used to treat patients who lack these cells as a result of specific disease conditions or as a result of high dose chemotherapy for treatment of malignancy (George, *Stem Cells 12*(Suppl 1):249-255, 1994 which is incorporated by reference). In the case of cell transplant after chemotherapy, autologous transplants can be performed by removing bone marrow cells before chemotherapy, expanding the cells *ex vivo* using methods that also function to purge malignant cells, and transplanting the expanded cells back into the patient following chemotherapy (for review, see Rummel and Van Zant, *J. Hematotherapy 3*:213-218, 1994 which is incorporated by reference). Since FGF-21 is expressed in liver cells, it is believed that FGF-21 can function to prevent or slow the progression of cirrhosis changes in liver cells, and to promote hepatic cell regeneration after injury or after surgical removal of part of the liver due to disease.

In a number of circumstances it would be desirable to determine the levels of FGF-21 in a patient. The identification of FGF-21 along with the present report showing expression of FGF-21 provides the basis for the conclusion that the presence of FGF-21 serves a normal physiological function related to cell growth and

survival. Endogenously produced FGF-21 may also play a role in certain disease conditions.

Given that FGF-21 is expressed in liver, thymic and testicular tissue, it is likely that the level of FGF-21 may be altered in a variety of conditions and that quantification of FGF-21 levels would provide clinically useful information. Furthermore, in the treatment of degenerative conditions, altered physiological function or in recovery from injury to the liver, testis or thymic cells, compositions containing FGF-21 can be administered and it would likely be desirable to achieve certain target levels of FGF-21 in sera or in any desired tissue compartment. It would, therefore, be advantageous to be able to monitor the levels of FGF-21 in a patient. Accordingly, the present invention also provides methods for detecting the presence of FGF-21 in a sample from a patient.

The term "detection" as used herein in the context of detecting the presence of FGF-21 in a patient is intended to include determining the amount of FGF-21 or the ability to express an amount of FGF-21 in a patient, distinguishing FGF-21 from other growth factors, the estimation of prognosis in terms of probable outcome of a degenerative disease and prospect for recovery, monitoring the FGF-21 levels over a period of time as a measure of status of the condition, and monitoring FGF-21 levels for determining a preferred therapeutic regimen for the patient.

To detect the presence of FGF-21 in a patient, a sample is obtained from the patient. The sample can be a tissue biopsy sample or a sample of blood, plasma, serum, CSF or the like. FGF-21 is expressed in liver tissues, as discussed in Example 2. Samples for detecting FGF-21 can be taken from this tissue. When assessing the levels of FGF-21 in the liver, thymus or testis, a preferred sample is a sample taken from these tissues or from veins draining these tissues.

In some instances it is desirable to determine whether the FGF-21 gene is intact in the patient or in a tissue or cell line within the patient. By an intact FGF-21 gene it is meant that there are no alterations in the gene such as point mutations, deletions, insertions, chromosomal breakage, chromosomal rearrangements and the like wherein such alteration might alter production of FGF-21 or alter its biological activity,

stability or the like to lead to disease processes or susceptibility to cellular degenerative conditions. Thus, in one embodiment of the present invention a method is provided for detecting and characterizing any alterations in the FGF-21 gene. The method comprises providing an oligonucleotide that contains the FGF-21 cDNA, genomic DNA or a fragment thereof or a derivative thereof. By a derivative of an oligonucleotide, it is meant that the derived oligonucleotide is substantially the same as the sequence from which it is derived in that the derived sequence has sufficient sequence complementarily to the sequence from which it is derived to hybridize to the FGF-21 gene. The derived nucleotide sequence is not necessarily physically derived from the nucleotide sequence, but may be generated in any manner including for example, chemical synthesis or DNA replication or reverse transcription or transcription.

Typically, patient genomic DNA is isolated from a cell sample from the patient and digested with one or more restriction endonucleases such as, for example, TaqI and AluI. Using the Southern blot protocol, which is well known in the art, this assay determines whether a patient or a particular tissue in a patient has an intact FGF-21 gene or an FGF-21 gene abnormality.

Hybridization to an FGF-21 gene would involve denaturing the chromosomal DNA to obtain a single-stranded DNA; contacting the single-stranded DNA with a gene probe associated with the FGF-21 gene sequence; and identifying the hybridized DNA-probe to detect chromosomal DNA containing at least a portion of a human FGF-21 gene.

The term "probe" as used herein refers to a structure comprised of a polynucleotide that forms a hybrid structure with a target sequence, due to complementarity of probe sequence with a sequence in the target region. Oligomers suitable for use as probes may contain a minimum of about 8-12 contiguous nucleotides which are complementary to the targeted sequence and preferably a minimum of about 20.

The FGF-21 gene probes of the present invention can be DNA or RNA oligonucleotides and can be made by any method known in the art such as, for example, excision, transcription or chemical synthesis. Probes may be labeled with any

detectable label known in the art such as, for example, radioactive or fluorescent labels or enzymatic marker. Labeling of the probe can be accomplished by any method known in the art such as by PCR, random priming, end labeling, nick translation or the like. One skilled in the art will also recognize that other methods not employing a labeled probe can be used to determine the hybridization. Examples of methods that can be used for detecting hybridization include Southern blotting, fluorescence in situ hybridization, and single-strand conformation polymorphism with PCR amplification.

Hybridization is typically carried out at 25° - 45° C, more preferably at 32° - 40° C and more preferably at 37° - 38° C. The time required for hybridization is from about 0.25 to about 96 hours, more preferably from about one to about 72 hours, and most preferably from about 4 to about 24 hours.

FGF-21 gene abnormalities can also be detected by using the PCR method and primers that flank or lie within the FGF-21 gene. The PCR method is well known in the art. Briefly, this method is performed using two oligonucleotide primers which are capable of hybridizing to the nucleic acid sequences flanking a target sequence that lies within an FGF-21 gene and amplifying the target sequence. The terms "oligonucleotide primer" as used herein refers to a short strand of DNA or RNA ranging in length from about 8 to about 30 bases. The upstream and downstream primers are typically from about 20 to about 30 base pairs in length and hybridize to the flanking regions for replication of the nucleotide sequence. The polymerization is catalyzed by a DNA-polymerase in the presence of deoxynucleotide triphosphates or nucleotide analogs to produce double-stranded DNA molecules. The double strands are then separated by any denaturing method including physical, chemical or enzymatic. Commonly, the method of physical denaturation is used involving heating the nucleic acid, typically to temperatures from about 80°C. to 105°C. for times ranging from about 1 to about 10 minutes. The process is repeated for the desired number of cycles.

The primers are selected to be substantially complementary to the strand of DNA being amplified. Therefore, the primers need not reflect the exact sequence of the template, but must be sufficiently complementary to selectively hybridize with the strand being amplified.

After PCR amplification, the DNA sequence comprising FGF-21 or pre-pro FGF-21 or a fragment thereof is then directly sequenced and analyzed by comparison of the sequence with the sequences disclosed herein to identify alterations which might change activity or expression levels or the like.

In another embodiment, a method for detecting FGF-21 is provided based upon an analysis of tissue expressing the FGF-21 gene. Certain tissues such as those identified below in Example 2 have been found to express the FGF-21 gene. The method comprises hybridizing a polynucleotide to mRNA from a sample of tissues that normally express the FGF-21 gene. The sample is obtained from a patient suspected of having an abnormality in the FGF-21 gene or in the FGF-21 gene of particular cells.

To detect the presence of mRNA encoding FGF-21 protein, a sample is obtained from a patient. The sample can be from blood or from a tissue biopsy sample. The sample may be treated to extract the nucleic acids contained therein. The resulting nucleic acid from the sample is subjected to gel electrophoresis or other size separation techniques.

The mRNA of the sample is contacted with a DNA sequence serving as a probe to form hybrid duplexes. The use of a labeled probes as discussed above allows detection of the resulting duplex.

When using the cDNA encoding FGF-21 protein or a derivative of the cDNA as a probe, high stringency conditions can be used in order to prevent false positives, that is the hybridization and apparent detection of FGF-21 nucleotide sequences when in fact an intact and functioning FGF-21 gene is not present. When using sequences derived from the FGF-21 cDNA, less stringent conditions could be used, however, this would be a less preferred approach because of the likelihood of false positives. The stringency of hybridization is determined by a number of factors during hybridization and during the washing procedure, including temperature, ionic strength, length of time and concentration of formamide. These factors are outlined in, for example, Sambrook *et al.*, Molecular Cloning: A Laboratory Manual, 2nd Ed. (1989) Cold Spring Harbor Press, Cold Spring Harbor, NY.

In order to increase the sensitivity of the detection in a sample of mRNA encoding the FGF-21 protein, the technique of reverse transcription/polymerization chain reaction (RT/PCR) can be used to amplify cDNA transcribed from mRNA encoding the FGF-21 protein. The method of RT/PCR is well known in the art, and can be performed as follows. Total cellular RNA is isolated by, for example, the standard guanidium isothiocyanate method and the total RNA is reverse transcribed. The reverse transcription method involves synthesis of DNA on a template of RNA using a reverse transcriptase enzyme and a 3′ end primer. Typically, the primer contains an oligo(dT) sequence. The cDNA thus produced is then amplified using the PCR method and FGF-21 specific primers. (Belyavsky *et al.*, *Nucl. Acid Res. 17*:2919-2932, 1989; Krug and Berger, *Methods in Enzymology*, *152*:316-325, Academic Press, NY, 1987 which are incorporated by reference).

The polymerase chain reaction method is performed as described above using two oligonucleotide primers that are substantially complementary to the two flanking regions of the DNA segment to be amplified.

Following amplification, the PCR product is then electrophoresed and detected by ethidium bromide staining or by phosphoimaging.

The present invention further provides for methods to detect the presence of the FGF-21 protein in a sample obtained from a patient. Any method known in the art for detecting proteins can be used. Such methods include, but are not limited to immunodiffusion, immunoelectrophoresis, immunochemical methods, binder-ligand assays, immunohistochemical techniques, agglutination and complement assays. (for example, see *Basic and Clinical Immunology*, 217-262, Sites and Terr, eds., Appleton & Lange, Norwalk, CT, 1991 which is incorporated by reference). Preferred are binder-ligand immunoassay methods including reacting antibodies with an epitope or epitopes of the FGF-21 protein and competitively displacing a labeled FGF-21 protein or derivative thereof. Preferred antibodies are prepared according to Example 4.

As used herein, a derivative of the FGF-21 protein is intended to include a polypeptide in which certain amino acids have been deleted or replaced or changed to modified or unusual amino acids wherein the FGF-21 derivative is biologically

equivalent to FGF-21 and wherein the polypeptide derivative cross-reacts with antibodies raised against the FGF-21 protein. By cross-reaction it is meant that an antibody reacts with an antigen other than the one that induced its formation.

Numerous competitive and non-competitive protein binding immunoassays are well known in the art. Antibodies employed in such assays may be unlabeled, for example as used in agglutination tests, or labeled for use in a wide variety of assay methods. Labels that can be used include radionuclides, enzymes, fluorescers, chemiluminescers, enzyme substrates or co-factors, enzyme inhibitors, particles, dyes and the like for use in radioimmunoassay (RIA), enzyme immunoassays, *e.g.*, enzyme-linked immunosorbent assay (ELISA), fluorescent immunoassays and the like.

Polyclonal or monoclonal antibodies to the FGF-21 protein or an epitope thereof can be made for use in immunoassays by any of a number of methods known in the art. By epitope reference is made to an antigenic determinant of a polypeptide. An epitope could comprise 3 amino acids in a spatial conformation which is unique to the epitope. Generally an epitope consists of at least 5 such amino acids. Methods of determining the spatial conformation of amino acids are known in the art, and include, for example, x-ray crystallography and 2 dimensional nuclear magnetic resonance.

One approach for preparing antibodies to a protein is the selection and preparation of an amino acid sequence of all or part of the protein, chemically synthesizing the sequence and injecting it into an appropriate animal, usually a rabbit or a mouse (see Example 4).

Oligopeptides can be selected as candidates for the production of an antibody to the FGF-21 protein based upon the oligopeptides lying in hydrophilic regions, which are thus likely to be exposed in the mature protein. Preferred oligopeptides are RQRYLYTDDAQQTEAH (residues 46-61 of SEQ NO:4) and HLPGNKSPHRDPAPR (residues 146-160 of SEQ ID NO:4). Additional oligopeptides can be determined using, for example, the Antigenicity Index of Welling, G.W. et al., FEBS Lett. 188:215-218, 1985, incorporated herein by reference.

Antibodies to FGF-21 can also be raised against oligopeptides that include one or more of the conserved regions identified herein such that the antibody

can cross-react with other family members. Such antibodies can be used to identify and isolate the other family members.

Methods for preparation of the FGF-21 protein or an epitope thereof include, but are not limited to chemical synthesis, recombinant DNA techniques or isolation from biological samples. Chemical synthesis of a peptide can be performed, for example, by the classical Merrifeld method of solid phase peptide synthesis (Merrifeld, *J. Am. Chem. Soc. 85*:2149, 1963 which is incorporated by reference) or the FMOC strategy on a Rapid Automated Multiple Peptide Synthesis system (E. I. du Pont de Nemours Company, Wilmington, DE) (Caprino and Han, *J. Org. Chem. 37*:3404, 1972 which is incorporated by reference).

Polyclonal antibodies can be prepared by immunizing rabbits or other animals by injecting antigen followed by subsequent boosts at appropriate intervals. The animals are bled and sera assayed against purified FGF-21 protein usually by ELISA or by bioassay based upon the ability to block the action of FGF-21 on liver or other cells. When using avian species, *e.g.*, chicken, turkey and the like, the antibody can be isolated from the yolk of the egg. Monoclonal antibodies can be prepared after the method of Milstein and Kohler by fusing splenocytes from immunized mice with continuously replicating tumor cells such as myeloma or lymphoma cells. (Milstein and Kohler, *Nature 256*:495-497, 1975; Gulfre and Milstein, *Methods in Enzymology: Immunochemical Techniques 73*:1-46, Langone and Banatis eds., Academic Press, 1981 which are incorporated by reference). The hybridoma cells so formed are then cloned by limiting dilution methods and supernates assayed for antibody production by ELISA, RIA or bioassay.

The unique ability of antibodies to recognize and specifically bind to target proteins provides an approach for treating an overexpression of the protein. Thus, another aspect of the present invention provides for a method for preventing or treating diseases involving overexpression of the FGF-21 protein by treatment of a patient with specific antibodies to the FGF-21 protein.

Specific antibodies, either polyclonal or monoclonal, to the FGF-21 protein can be produced by any suitable method known in the art as discussed above.

For example, murine or human monoclonal antibodies can be produced by hybridoma technology or, alternatively, the FGF-21 protein, or an immunologically active fragment thereof, or an anti-idiotypic antibody, or fragment thereof can be administered to an animal to elicit the production of antibodies capable of recognizing and binding to the FGF-21 protein. Such antibodies can be from any class of antibodies including, but not limited to IgG, IgA, IgM, IgD, and IgE or in the case of avian species, IgY and from any subclass of antibodies.

Polypeptides encoded by the instant polynucleotides and corresponding full-length genes can be used to screen peptide libraries, protein libraries, small molecule libraries, and phage display libraries, and other known methods, to identify analogs or antagonists.

Native FGF polypeptides may play a role in cancer. For example, FGF family members can induce marked morphological transformation of NIH 3T3 cells, and exhibit strong tumorigenicity in nude mice. Angiogenic activity has been exhibited by FGF family members. Thus, inhibitors of FGF can be used to treat cancer, such as prostate cancer.

A library of peptides may be synthesized following the methods disclosed in U.S. Patent No. 5,010,175, and in PCT No. WO 91/17823. As described below in brief, a mixture of peptides is prepared, which is then screened to identify the peptides exhibiting the desired signal transduction and receptor binding activity. According to the method of the '175 patent, a suitable peptide synthesis support (*e.g.*, a resin) is coupled to a mixture of appropriately protected, activated amino acids. The concentration of each amino acid in the reaction mixture is balanced or adjusted in inverse proportion to its coupling reaction rate so that the product is an equimolar mixture of amino acids coupled to the starting resin. The bound amino acids are then deprotected, and reacted with another balanced amino acid mixture to form an equimolar mixture of all possible dipeptides. This process is repeated until a mixture of peptides of the desired length (*e.g.*, hexamers) is formed. Note that one need not include all amino acids in each step: one may include only one or two amino acids in some steps (*e.g.*, where it is known that a particular amino acid is essential in a given

position), thus reducing the complexity of the mixture. After the synthesis of the peptide library is completed, the mixture of peptides is screened for binding to the selected polypeptide. The peptides are then tested for their ability to inhibit or enhance activity. Peptides exhibiting the desired activity are then isolated and sequenced.

The method described in PCT No. WO 91/17823 is similar. However, instead of reacting the synthesis resin with a mixture of activated amino acids, the resin is divided into twenty equal portions (or into a number of portions corresponding to the number of different amino acids to be added in that step), and each amino acid is coupled individually to its portion of resin. The resin portions are then combined, mixed, and again divided into a number of equal portions for reaction with the second amino acid. In this manner, each reaction may be easily driven to completion. Additionally, one may maintain separate "subpools" by treating portions in parallel, rather than combining all resins at each step. This simplifies the process of determining which peptides are responsible for any observed receptor binding or signal transduction activity.

In such cases, the subpools containing, *e.g.*, 1-2,000 candidates each are exposed to one or more polypeptides of the invention. Each subpool that produces a positive result is then resynthesized as a group of smaller subpools (sub-subpools) containing, *e.g.*, 20-100 candidates, and reassayed. Positive sub-subpools may be resynthesized as individual compounds, and assayed finally to determine the peptides that exhibit a high binding constant. These peptides can be tested for their ability to inhibit or enhance the native activity. The methods described in PCT No. WO 91/7823 and U.S. Patent No. 5,194,392 (herein incorporated by reference) enable the preparation of such pools and subpools by automated techniques in parallel, such that all synthesis and resynthesis may be performed in a matter of days.

Peptide agonists or antagonists are screened using any available method, such as signal transduction, antibody binding, receptor binding and mitogenic assays. The assay conditions ideally should resemble the conditions under which the native activity is exhibited *in vivo*, that is, under physiologic pH, temperature, and ionic strength. Suitable agonists or antagonists will exhibit strong inhibition or enhancement

of the native activity at concentrations that do not cause toxic side effects in the subject. Agonists or antagonists that compete for binding to the native polypeptide may require concentrations equal to or greater than the native concentration, while inhibitors capable of binding irreversibly to the polypeptide may be added in concentrations on the order of the native concentration.

The availability of hFGF-21 and mFGF-21 allows for the identification of small molecules and low molecular weight compounds that inhibit the binding of FGF-21 to its receptor, through routine application of high-throughput screening methods (HTS). HTS methods generally refer to technologies that permit the rapid assaying of lead compounds for therapeutic potential. HTS techniques employ robotic handling of test materials, detection of positive signals, and interpretation of data. Lead compounds may be identified via the incorporation of radioactivity or through optical assays that rely on absorbance, fluorescence or luminescence as read-outs. Gonzalez, J.E. et al., (1998) *Curr. Opin. Biotech. 9*:624-631. Assays for detecting interaction between an FGF molecule and FGF receptor are described in, for example, Blunt, A. G. et al., (1997) *J. Biol. Chem. 272*:3733-3738, and such assays can be adapted for determining if a candidate molecule can inhibit the interaction between FGF-21 and its receptor.

Model systems are available that can be adapted for use in high throughput screening for compounds that inhibit the interaction of FGF-21 with its receptor, for example by competing with FGF-21 for receptor binding. Sarubbi et al., (1996) *Anal. Biochem. 237*:70-75 describe cell-free, non-isotopic assays for identifying molecules that compete with natural ligands for binding to the active site of IL-1 receptor. Martens, C. et al., (1999) *Anal. Biochem. 273*:20-31 describe a generic particle-based nonradioactive method in which a labeled ligand binds to its receptor immobilized on a particle; label on the particle decreases in the presence of a molecule that competes with the labeled ligand for receptor binding.

The therapeutic FGF-21 polynucleotides and polypeptides of the present invention may be utilized in gene delivery vehicles. The gene delivery vehicle may be of viral or non-viral origin (*see generally*, Jolly, *Cancer Gene Therapy 1*:51-64 (1994);

Kimura, *Human Gene Therapy 5*:845-852 (1994); Connelly, *Human Gene Therapy 1*:185-193 (1995); and Kaplitt, *Nature Genetics 6*:148-153 (1994)). Gene therapy vehicles for delivery of constructs including a coding sequence of a therapeutic of the invention can be administered either locally or systemically. These constructs can utilize viral or non-viral vector approaches. Expression of such coding sequences can be induced using endogenous mammalian or heterologous promoters. Expression of the coding sequence can be either constitutive or regulated.

The present invention can employ recombinant retroviruses which are constructed to carry or express a selected nucleic acid molecule of interest. Retrovirus vectors that can be employed include those described in EP 0 415 731; WO 90/07936; WO 94/03622; WO 93/25698; WO 93/25234; U.S. Patent No. 5,219,740; WO 93/11230; WO 93/10218; Vile and Hart, *Cancer Res. 53*:3860-3864 (1993); Vile and Hart, *Cancer Res. 53*:962-967 (1993); Ram *et al.*, *Cancer Res. 53*:83-88 (1993); Takamiya *et al.*, *J. Neurosci. Res. 33*:493-503 (1992); Baba *et al.*, *J. Neurosurg. 79*:729-735 (1993); U.S. Patent No. 4,777,127; GB Patent No. 2,200,651; and EP 0 345 242. Preferred recombinant retroviruses include those described in WO 91/02805.

Packaging cell lines suitable for use with the above-described retroviral vector constructs may be readily prepared (see PCT publications WO 95/30763 and WO 92/05266), and used to create producer cell lines (also termed vector cell lines) for the production of recombinant vector particles. Within particularly preferred embodiments of the invention, packaging cell lines are made from human (such as HT1080 cells) or mink parent cell lines, thereby allowing production of recombinant retroviruses that can survive inactivation in human serum.

The present invention also employs alphavirus-based vectors that can function as gene delivery vehicles. Such vectors can be constructed from a wide variety of alphaviruses, including, for example, Sindbis virus vectors, Semliki forest virus (ATCC VR-67; ATCC VR-1247), Ross River virus (ATCC VR-373; ATCC VR-1246) and Venezuelan equine encephalitis virus (ATCC VR-923; ATCC VR-1250; ATCC VR 1249; ATCC VR-532). Representative examples of such vector systems include those described in U.S. Patent Nos. 5,091,309; 5,217,879; and 5,185,440; and PCT

Publication Nos. WO 92/10578; WO 94/21792; WO 95/27069; WO 95/27044; and WO 95/07994.

Gene delivery vehicles of the present invention can also employ parvovirus such as adeno-associated virus (AAV) vectors. Representative examples include the AAV vectors disclosed by Srivastava in WO 93/09239, Samulski *et al.*, *J. Vir. 63*:3822-3828 (1989); Mendelson *et al.*, *Virol. 166*:154-165 (1988); and Flotte *et al.*, *P.N.A.S. 90*:10613-10617 (1993).

Representative examples of adenoviral vectors include those described by Berkner, *Biotechniques 6*:616-627 (Biotechniques); Rosenfeld *et al.*, *Science 252*:431-434 (1991); WO 93/19191; Kolls *et al.*, *P.N.A.S.* :215-219 (1994); Kass-Eisler *et al.*, *P.N.A.S. 90*:11498-11502 (1993); Guzman *et al.*, *Circulation 88*:2838-2848 (1993); Guzman *et al.*, *Cir. Res. 73*:1202-1207 (1993); Zabner *et al.*, *Cell 75*:207-216 (1993); Li *et al.*, *Hum. Gene Ther. 4*:403-409 (1993); Cailaud *et al.*, *Eur. J. Neurosci. 5*:1287-1291 (1993); Vincent *et al.*, *Nat. Genet. 5*:130-134 (1993); Jaffe *et al.*, *Nat. Genet. 1*:372-378 (1992); and Levrero *et al.*, *Gene 101*:195-202 (1992). Exemplary adenoviral gene therapy vectors employable in this invention also include those described in WO 94/12649, WO 93/03769; WO 93/19191; WO 94/28938; WO 95/11984 and WO 95/00655. Administration of DNA linked to killed adenovirus as described in Curiel, *Hum. Gene Ther. 3*:147-154 (1992) may be employed.

Other gene delivery vehicles and methods may be employed, including polycationic condensed DNA linked or unlinked to killed adenovirus alone, for example Curiel, *Hum. Gene Ther. 3*:147-154 (1992); ligand-linked DNA, for example see Wu, *J. Biol. Chem. 264*:16985-16987 (1989); eukaryotic cell delivery vehicles cells, for example see U.S. Serial No. 08/240,030, filed May 9, 1994, and U.S. Serial No. 08/404,796; deposition of photopolymerized hydrogel materials; hand-held gene transfer particle gun, as described in U.S. Patent No. 5,149,655; ionizing radiation as described in U.S. Patent No. 5,206,152 and in WO 92/11033; nucleic charge neutralization or fusion with cell membranes. Additional approaches are described in Philip, *Mol. Cell Biol. 14*:2411-2418 (1994), and in Woffendin, *Proc. Natl. Acad. Sci. 91*:1581-1585 (1994).

Naked DNA may also be employed. Exemplary naked DNA introduction methods are described in WO 90/11092 and U.S. Patent No. 5,580,859. Uptake efficiency may be improved using biodegradable latex beads. DNA coated latex beads are efficiently transported into cells after endocytosis initiation by the beads. The method may be improved further by treatment of the beads to increase hydrophobicity and thereby facilitate disruption of the endosome and release of the DNA into the cytoplasm. Liposomes that can act as gene delivery vehicles are described in U.S. Patent No. 5,422,120, PCT Patent Publication Nos. WO 95/13796, WO 94/23697, and WO 91/14445, and EP No. 0 524 968.

Further non-viral delivery suitable for use includes mechanical delivery systems such as the approach described in Woffendin *et al.*, *Proc. Natl. Acad. Sci. USA* *91*(24):11581-11585 (1994). Moreover, the coding sequence and the product of expression of such can be delivered through deposition of photopolymerized hydrogel materials. Other conventional methods for gene delivery that can be used for delivery of the coding sequence include, for example, use of hand-held gene transfer particle gun, as described in U.S. Patent No. 5,149,655; use of ionizing radiation for activating transferred gene, as described in U.S. Patent No. 5,206,152 and PCT Patent Publication No. WO 92/11033.

FGF has been implicated in diseases characterized by loss of function, inadequate function/number, abnormal function or death of cells, tissues or organs for which function or survival can be prolonged/rescued, and abnormalities reversed or prevented by therapy with FGF.

Loss of pulmonary, bronchia or alveolar cells or function, healing of pulmonary or bronchia wounds, pulmonary infraction, emphysema/chronic obstructive pulmonary disease, asthma, sequelae of infectious or autoimmune disease, sequelae of pulmonary arterial or venous hypertension, pulmonary fibrosis, pulmonary disease of immaturity, and cystic fibrosis are conditions amenable to treatment with FGF.

Ischemic vascular disease may be amenable to FGF-21 treatment, wherein the disease is characterized by inadequate blood flow to an organ(s). Treatment may induce therapeutic angiogenesis or preserve function/survival of cells

(myocardial ischemia/infarction, peripheral vascular disease, renal artery disease, stroke). Cardiomyopathies characterized by loss of function or death of cardiac myocytes or supporting cells in the heart (congestive heart failure, myocarditis) may also be treated using FGF-21, as can musculoskeletal disease characterized by loss of function, inadequate function or death of skeletal muscle cells, bone cells or supporting cells. Examples include skeletal myopathies, bone disease, and arthritis.

FGF-21 polynucleotides and polypeptides may aid in correction of congenital defects due to loss of FGF-21 molecule or its function (liver, heart, lung, brain, limbs, kidney, etc.).

Treatment of wound healing is yet another use of FGF-21 polypeptides and polynucleotides, either due to trauma, disease, medical or surgical treatment, including regeneration of cell populations and tissues depleted by these processes. Examples include liver regeneration, operative wound healing, re-endothelialization of injured blood vessels, healing of traumatic wounds, healing of ulcers due to vascular, metabolic disease, etc., bone fractures, loss of cells due to inflammatory disease, etc.

FGF-21 may also be used in screens to identify drugs for treatment of cancers which involve over activity of the molecule, or new targets which would be useful in the identification of new drugs.

For all of the preceding embodiments, the clinician will determine, based on the specific condition, whether FGF-21 polypeptides or polynucleotides, antibodies to FGF-21, or small molecules such as peptide analogues or antagonists, will be the most suitable form of treatment. These forms are all within the scope of the invention.

Preferred embodiments of the invention are described in the following examples. Other embodiments within the scope of the claims herein will be apparent to one skilled in the art from consideration of the specification or practice of the invention as disclosed herein. It is intended that the specification, together with the examples, be considered exemplary only, with the scope and spirit of the invention being indicated by the claims which follow the examples.

EXAMPLES

EXAMPLE 1

Isolation and Analysis of Mouse FGF-21 --- DNA was prepared from mouse embryo cDNA. DNA was amplified from mouse embryo cDNA by polymerase chain reaction (PCR) for 30 cycles in 25 μl of a reaction mixture containing each of the sense and antisense degenerate primers representing all possible codons corresponding to the amino acid sequences of human FGF-19, RPYDGYN and LPMLPM, respectively. The amplified product was further amplified by PCR with each of the sense and antisense degenerate primers representing all possible codons corresponding to the amino acid sequences of human FGF-19, RPDGYN and HFLPML, respectively. The amplified DNAs of expected size (approximately 120 base pairs) were cloned. By determination of the nucleotide sequences of the cloned DNAs, a novel mouse FGF, FGF-21, cDNA was identified. To determine the entire coding region of the novel FGF cDNA, the coding region was amplified from mouse embryo cDNA by adaptor-ligation mediated PCR using a Marathon cDNA amplification kit (Clontech, Palo Alto, California) and primers specific for the FGF. The cDNA encoding the entire coding region of the FGF was amplified from mouse embryo cDNA by PCR using the FGF-specific primers including the 5′ and 3′ noncoding sequences, and cloned into the pGEM-T DNA vector. The nucleotide sequence is shown in SEQ ID NO:1 and the amino acid sequence is shown in SEQ ID NO:2.

EXAMPLE 2

Expression of FGF-21 in Mouse Tissues --- Poly $(A)^+$ RNA (10 μg) from mouse tissues was dissolved on a denaturing agarose gel (1%) containing formaldehyde, and transferred to a nitrocellulose membrane in 20X SSC (1X SSC:0.15 M NACl/0.015 M sodium citrate) overnight. A ^{32}P-labeled FGF-21 cDNA probe (~650 base pairs) was labeled with a random primer labeling kit (Pharmacia Biotech, Uppsala,

Sweden) and deoxycytidine 5′-[α-^{32}P-] triphosphate (~110 TBq/mmol) (ICN Biomedicals Inc., Costa Mesa, California). The membrane was incubated in hybridization solution containing the labeled probe as described (Hoshikawa et al., *Biochem. Biophys. Res. Commun. 244*:187-191 (1998)), and analyzed with a radio-imaging analyzer (BAS 2000, Fuji Photo Film Co., Tokyo, Japan). As shown in Figure 3, FGF-21 expression was most predominant in liver, with expression also seen in testis and thymus.

EXAMPLE 3

Isolation and Analysis of Human FGF-21 --- The human FGF-21 gene was located in the 5′ flanking region of a putative human alpha 1,2-fucosyltransferase gene. The cDNA encoding the entire coding region of human FGF-21 was amplified from fetal brain cDNA by PCR using the FGF-specific primers including the 5′ and 3′ noncoding sequences, and cloned into the pGEM-T DNA vector. The protein contains 209 amino acids, as shown in SEQ ID NO:4 (Figure 5), and is encoded by the polynucleotide sequence of SEQ ID NO:3. Primers for amplification of human FGF-21 cDNA coding region are: sense primer for FGF-21: 5′ agccattgatggactcggac 3′; antisense primer for FGF-21: 5′ tggcttcaggaagcgtagct 3′.

EXAMPLE 4

Preparation of Antisera to FGF-21 by Immunization of Rabbits with an FGF-21 Peptide --- A peptide sequence corresponding to selected contiguous amino acids of the human FGF-21 protein is synthesized and coupled to keyhole limpet hemocyanin (KLH) as described (Harlow and Land, Antibodies: A Laboratory Manual, 1988. Cold Spring Harbor Laboratory, New York, NY) The KLH-coupled peptide is used to immunize rabbits. Antisera are tested for specificity to FGF-21, and for cross-reactivity with other FGF proteins.

Exemplary peptide sequences are:

1. RQRYLYDDAQQTEAH (residues 46-61 of SEQ ID NO:4)
2. HLPGNKSPHRDPAPR (residues 146-160 of SEQ ID NO:4)

All patents, published patent applications and publications cited herein are incorporated by reference as if set forth fully herein.

Although certain preferred embodiments have been described herein, it is not intended that such embodiments be construed as limitations on the scope of the invention except as set forth in the following claims.

CLAIMS

WE CLAIM:

1. An isolated nucleic acid molecule comprising a polynucleotide selected from the group consisting of:

(a) a polynucleotide encoding amino acids from about 1 to about 209 of SEQ ID NO:4;

(b) a polynucleotide encoding amino acids from about 2 to about 209 of SEQ ID NO:4;

(c) a polynucleotide encoding amino acids from about 1 to about 177 of SEQ ID NO:4;

(d) a polynucleotide encoding amino acids from about 40 to about 209 of SEQ ID NO:4;

(e) a polynucleotide encoding amino acids from about 40 to about 177 of SEQ ID NO:4;

(f) the polynucleotide complement of (a), (b), (c), (d) or (e); and

(g) a polynucleotide at least 90% identical to the polynucleotide of (a), (b), (c), (d) or (e).

2. An isolated nucleic acid molecule which comprises 20-600 contiguous nucleotides from the coding region of SEQ ID NO:3.

3. The isolated nucleic acid molecule of claim 2, which comprises 60-400 contiguous nucleotides from the coding region of SEQ ID NO:3

4. The isolated nucleic acid molecule of claim 3, which comprises 200-300 contiguous nucleotides from the coding region of SEQ ID NO:3.

5. An isolated nucleic acid molecule comprising a polynucleotide encoding a polypeptide wherein, except for at least one conservative amino acid substitution, said polypeptide has an amino acid sequence selected from the group consisting of:

(a) amino acids from about 1 to about 209 of SEQ ID NO:4;

(b) amino acids from about 2 to about 209 of SEQ ID NO:4;

(c) amino acids from about 1 to about 177 of SEQ ID NO:4;

(d) amino acids from about 40 to about 209 of SEQ ID NO:4; and

(e) amino acids from about 40 to about 177 of SEQ ID NO:4.

6. The isolated nucleic acid molecule of claim 1, which is DNA.

7. A method of making a recombinant vector comprising inserting a nucleic acid molecule of claim 1 into a vector in operable linkage to a promoter.

8. A recombinant vector produced by the method of claim 7.

9. A method of making a recombinant host cell comprising introducing the recombinant vector of claim 8 into a host cell.

10. A recombinant host cell produced by the method of claim 9.

11. A recombinant method of producing a polypeptide, comprising culturing the recombinant host cell of claim 10 under conditions such that said polypeptide is expressed and recovering said polypeptide.

12. An isolated polypeptide comprising amino acids at least 95% identical to amino acids selected from the group consisting of:

(a) amino acids from about 1 to about 209 of SEQ ID NO:4;

(b) amino acids from about 2 to about 209 of SEQ ID NO:4;

(c) amino acids from about 1 to about 177 of SEQ ID NO:4;

(d) amino acids from about 40 to about 209 of SEQ ID NO:4; and

(e) amino acids from about 40 to about 177 of SEQ ID NO:4.

13. An isolated polypeptide wherein, except for at least one conservative amino acid substitution, said polypeptide has an amino acid sequence selected from the group consisting of:

(a) amino acids from about 1 to about 209 of SEQ ID NO:4;

(b) amino acids from about 2 to about 209 of SEQ ID NO:4;

(c) amino acids from about 1 to about 177 of SEQ ID NO:4;

(d) amino acids from about 40 to about 209 of SEQ ID NO:4; and

(e) amino acids from about 40 to about 177 of SEQ ID NO:4.

14. An isolated polypeptide comprising amino acids selected from the group consisting of:

(a) amino acids from about 1 to about 209 of SEQ ID NO:4;

(b) amino acids from about 2 to about 209 of SEQ ID NO:4;

(c) amino acids from about 1 to about 177 of SEQ ID NO:4;

(d) amino acids from about 40 to about 209 of SEQ ID NO:4; and

(e) amino acids from about 40 to about 177 of SEQ ID NO:4.

15. An epitope-bearing portion of the polypeptide of SEQ ID NO:4.

16. The epitope-bearing portion of claim 15, which comprises between 10 and 50 contiguous amino acids of SEQ ID NO:4.

17. The epitope-bearing portion of claim 15, which comprises amino acids RQRYLYTDDAQQTEAH (SEQ ID NO:7).

18. The epitope-bearing portion of claim 15, which comprises amino

acids HLPGNKSPHRDPAPR (SEQ ID NO:8).

19. An isolated antibody that binds specifically to the polypeptide of claim 12.

20. An isolated antibody that binds specifically to the polypeptide of claim 13.

21. An isolated antibody that binds specifically to the polypeptide of claim 14.

22. A pharmaceutical composition comprising the polypeptide of claim 12, in combination with a pharmaceutically acceptable carrier.

23. A method for providing trophic support for cells in a patient in need thereof, the method comprising administering to the patient a composition comprising a polynucleotide encoding the polypeptide of SEQ ID NO:4.

24. The method of claim 23 wherein said polynucleotide is administered by implanting cells which express said polynucleotide into the patient, wherein said cells express FGF-21 polypeptide in the patient.

25. The method of claim 23 wherein the implanted cells are encapsulated in a semipermeable membrane.

26. The method of claim 23 wherein the patient suffers from a condition characterized by inadequate numbers of hepatic cells.

27. The method of claim 23 wherein the condition is cirrhosis of the liver.

28. The method of claim 23 wherein said patient suffers from a condition characterized by inadequate function or number of testicular cells.

29. The method of claim 28 wherein said condition is at least one condition selected from the group consisting of infertility, impotence, and testicular cancer.

30. The method of claim 23 wherein the patient suffers from a condition characterized by inadequate function of the thymus.

31. The method of claim 30 wherein said condition is at least one condition selected from the group consisting of leukemia, lymphoma, autoimmune disease, proliferative disorder of the thymus, and differentiation disorder of the thymus.

32. A method for providing trophic support for cells in a patient in need thereof, the method comprising administering to the patient a composition comprising a polypeptide of SEQ ID NO:4.

33. The method of claim 28 wherein the patient suffers from a condition characterized by inadequate numbers of hepatic cells.

34. The method of claim 29 wherein the condition is cirrhosis of the liver.

35. A method of alleviating a disease condition in the liver of a human patient wherein said disease condition is alleviated by at least one method selected from the group consisting of slowing degeneration of, restoring function of, and increasing the number of, functional hepatic cells in said human patient, said method comprising administering to said patient a pharmaceutically effective

composition comprising a polypeptide having the amino acid sequence of SEQ ID NO:4.

36. A method of alleviating a disease condition in the thymus of a human patient wherein said disease condition is alleviated by at least one method selected from the group consisting of preventing degeneration of, slowing degeneration of, increasing the number of, functional thymic cells in said human patient, said method comprising administering to said patient a pharmaceutically effective composition comprising a polypeptide having the amino acid sequence of SEQ ID NO:4.

37. A method of alleviating a disease condition in the testis of a human patient wherein said disease condition is alleviated by at least one method selected from the group consisting of preventing degeneration of, slowing degeneration of, and increasing the number of, functional testicular cells in said human patient, said method comprising administering to said patient a pharmaceutically effective composition comprising a polypeptide having the amino acid sequence of SEQ ID NO:4.

38. A kit for detecting the presence of mRNA encoding FGF-21 in a sample from a patient, said kit comprising a polynucleotide having at least 20 contiguous nucleotides of the polynucleotide of claim 3, packaged in a container.

39. The kit according to claim 38 wherein the polynucleotide encodes at least six contiguous amino acids of SEQ ID NO:4.

40. A kit for detecting the presence of FGF-21 polypeptide in a sample from a patient, said kit comprising an antibody according to claim 19, packaged in a container.

41. An isolated nucleic acid molecule comprising a polynucleotide

selected from the group consisting of:

(a) a polynucleotide encoding amino acids from about 1 to about 210 of SEQ ID NO:2;

(b) a polynucleotide encoding amino acids from about 2 to about 21 of SEQ ID NO:2;

(c) a polynucleotide encoding amino acids from about 1 to about 177 of SEQ ID NO:2;

(d) a polynucleotide encoding amino acids from about 40 to about 210 of SEQ ID NO:2;

(e) a polynucleotide encoding amino acids from about 40 to about 177 of SEQ ID NO:2;

(f) the polynucleotide complement of (a), (b), (c), (d) or (e); and

(h) a polynucleotide at least 90% identical to the polynucleotide of (a), (b), (c), (d) or (e).

42. An isolated nucleic acid molecule which comprises 20-600 contiguous nucleotides from the coding region of SEQ ID NO:1.

43. The isolated nucleic acid molecule of claim 42, which comprises 60-400 contiguous nucleotides from the coding region of SEQ ID NO:1.

44. The isolated nucleic acid molecule of claim 43, which comprises 200-300 contiguous nucleotides from the coding region of SEQ ID NO:1.

45. An isolated nucleic acid molecule comprising a polynucleotide encoding a polypeptide wherein, except for at least one conservative amino acid substitution, said polypeptide has an amino acid sequence selected from the group consisting of:

(a) amino acids from about 1 to about 210 of SEQ ID NO:2;

(b) amino acids from about 2 to about 210 of SEQ ID NO:2;

(c) amino acids from about 1 to about 177 of SEQ ID NO:2;

(d) amino acids from about 40 to about 210 of SEQ ID NO:2; and

(e) amino acids from about 40 to about 177 of SEQ ID NO:4.

46. The isolated nucleic acid molecule of claim 41, which is DNA.

47. A method of making a recombinant vector comprising inserting a nucleic acid molecule of claim 41 into a vector in operable linkage to a promoter.

48. A recombinant vector produced by the method of claim 47.

49. A method of making a recombinant host cell comprising introducing the recombinant vector of claim 48 into a host cell.

50. A recombinant host cell produced by the method of claim 49.

51. A recombinant method of producing a polypeptide, comprising culturing the recombinant host cell of claim 50 under conditions such that said polypeptide is expressed and recovering said polypeptide.

52. An isolated polypeptide comprising amino acids at least 95% identical to amino acids selected from the group consisting of:

(a) amino acids from about 1 to about 210 of SEQ ID NO:2;

(b) amino acids from about 2 to about 210 of SEQ ID NO:2;

(c) amino acids from about 1 to about 177 of SEQ ID NO:2;

(d) amino acids from about 40 to about 210 of SEQ ID NO:2; and

(e) amino acids from about 40 to about 177 of SEQ ID NO:2.

53. An isolated polypeptide wherein, except for at least one conservative amino acid substitution, said polypeptide has an amino acid sequence

selected from the group consisting of:

(a) amino acids from about 1 to about 210 of SEQ ID NO:2;

(b) amino acids from about 2 to about 210 of SEQ ID NO:2;

(c) amino acids from about 1 to about 177 of SEQ ID NO:2;

(d) amino acids from about 40 to about 210 of SEQ ID NO:2; and

(e) amino acids from about 40 to about 177 of SEQ ID NO:2.

54. An isolated polypeptide comprising amino acids selected from the group consisting of:

(a) amino acids from about 1 to about 210 of SEQ ID NO:2;

(b) amino acids from about 2 to about 210 of SEQ ID NO:2;

(c) amino acids from about 1 to about 177 of SEQ ID NO:2;

(d) amino acids from about 40 to about 210 of SEQ ID NO:2; and

(e) amino acids from about 40 to about 177 of SEQ ID NO:2.

55. An epitope-bearing portion of the polypeptide of SEQ ID NO:2.

56. The epitope-bearing portion of claim 55, which comprises between 10 and 50 contiguous amino acids of SEQ ID NO:2.

57. An isolated antibody that binds specifically to the polypeptide of claim 52.

58. An isolated antibody that binds specifically to the polypeptide of claim 53.

59. An isolated antibody that binds specifically to the polypeptide of claim 54.

```
human FGF-21        MDSDETGFEHSGLWVSVLAGLLLGACQAHPIPDSSPLLQFG-GQV-RQRYLYTD  52
                              **  *  *  *   *     *  **   * *   *  ***
mouse FGF-15  MARKWNGRAVARALVLATLWLAVS-GRPLAQ-QSQSVSDEDPLFLYGWGKITRLQYLYSA  58

              DAQQTEAHLEIREDGTVGGAADQSPESLLQLKALKPGVIQILGVKTSRFLCQRPDGALYG 112
                    * ** ** *    **    **   *     * *  *   * **   ** **
              GPYVSNCFLRIRSDGSVDCEEDQNERNLLEFRAVALKTIAIKDVSSVRYLCMSADGKIYG 118

              SLHFDPEACSFRELLLEDGYNVYQSEAHGLPLHLPGNKSPHRDPAPRGPARFLPLPGLPP 172
                   * * ***     *** * *  * *       * *        *  * *
              LIRYSEEDCTFREEMDCLGYNQYRSMKHHLHIIFIQAK-PREQLQDQKPSNFIPVFHRSF 177

              ALPEPPGILAPQ--PPDVGSSDPLSMVGPSQG--RSPSYAS
                           *    * **  **        ***
              FETGDQLRSKMFSLPLESDSMDPFRMVEDVDHLVKSPSFQK
```

Fig. 1

```
Human FGF-21    MDSDETGFEHSGLWVSVLAGLLLG-ACQAHPIPDSSPLLQF--GGQVRQRYLYTDDAQQ-   56
                         *   **** * *       * *      *   * * ***
Human FGF-19      MRSGCVVVHVW--ILAGLWLAVAGRPLAFSDAGPHVHYGWGDPIRLRHLYTSGPHGL   55

                  TEAHLEIREDGTVGGAADQSPESLLQLKALKPGVIQILGVKTSRFLCQRPDGALYGSLHF  116
                     * ** ** *  *  **  *** **       * **   * **   **   * *
                  SSCFLRIRADGVVDCARGQSAHSLLEIKAVALRTVAIKGVHSVRYLCMGADGKMQGLLQY  115

                  DPEACSFRELLLEDGYNVYQSEAHGLPLHLPGNKSPH--RDPAPRGPARFLPLPGLPPAL  174
                    * * * *    ****** ** * **  *   *                ***     *
                  SEEDCAFEEEIRPDGYNVYRSEKHRLPVSLSSAKQRQLYKNRGFLPLSHFLPMLPMVPEE  175

                  PEP-PGILAPQ----PPDVGSSDPLSMV-GPSQGRSPSYAS  209
                  **   * *       *    * **   * *    ****
                  PEDLRGHLESDMFSSPLETDSMDPFGLVTGLEAVRSPSFEK  216
```

Fig. 2

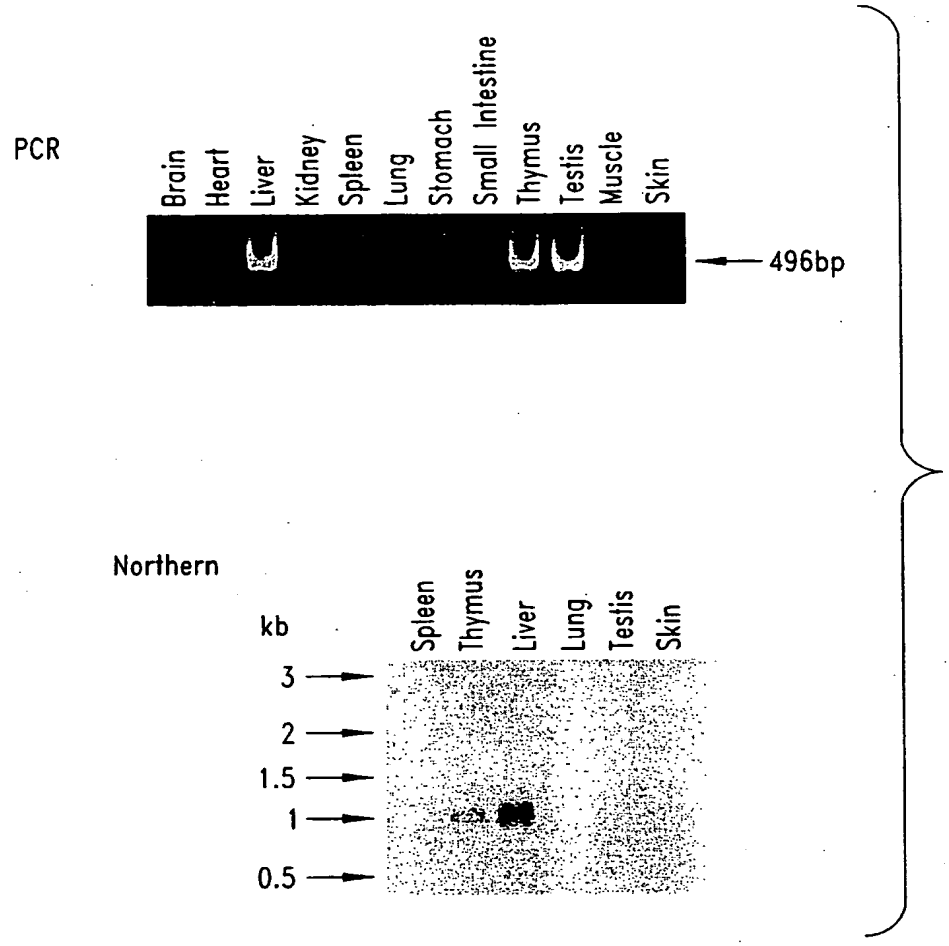
PCR
Brain
Heart
Liver
Kidney
Spleen
Lung
Stomach
Small Intestine
Thymus
Testis
Muscle
Skin
496bp
Northern
kb
3
2
1.5
1
0.5
Spleen
Thymus
Liver
Lung
Testis
Skin

Fig. 3

Filename: mouse FGF-21 cDNA in pGEM-T
Sequence Size: 659
Sequence Position: 1 - 659
Translation Position: 14 - 646

```
        10        20        30        40        50        60
GAGCGCAGCCCTGATGGAATGGATGAGATCTAGAGTTGGGACCCTGGGACTGTGGGTCCG  SEQ ID NO:1
            M  E  W  M  R  S  R  V  G  T  L  G  L  W  V  R   SEQ ID NO:2

        70        80        90       100       110       120
ACTGCTGCTGGCTGTCTTCCTGCTGGGGGTCTACCAAGCATACCCCATCCCTGACTCCAG
 L  L  L  A  V  F  L  L  G  V  Y  Q  A  Y  P  I  P  D  S  S

       130       140       150       160       170       180
CCCCCTCCTCCAGTTTGGGGGTCAAGTCCGGCAGAGGTACCTCTACACAGATGACGACCA
 P  L  L  Q  F  G  G  Q  V  R  Q  R  Y  L  Y  T  D  D  D  Q

       190       200       210       220       230       240
AGACACTGAAGCCCACCTGGAGATCAGGGAGGATGGAACAGTGGTAGGCGCAGCACACCG
 D  T  E  A  H  L  E  I  R  E  D  G  T  V  V  G  A  A  H  R

       250       260       270       280       290       300
CAGTCCAGAAAGTCTCCTGGAGCTCAAAGCCTTGAAGCCAGGGGTCATTCAAATCCTGGG
 S  P  E  S  L  L  E  L  K  A  L  K  P  G  V  I  Q  I  L  G

       310       320       330       340       350       360
TGTCAAAGCCTCTAGGTTTCTTTGCCAACAGCCAGATGGAGCTCTCTATGGATCGCCTCA
 V  K  A  S  R  F  L  C  Q  Q  P  D  G  A  L  Y  G  S  P  H

       370       380       390       400       410       420
CTTTGATCCTGAGGCCTGCAGCTTCAGAGAACTGCTGCTGGAGGACGGTTACAATGTGTA
 F  D  P  E  A  C  S  F  R  E  L  L  L  E  D  G  Y  N  V  Y

       430       440       450       460       470       480
CCAGTCTGAAGCCCATGGCCTGCCCCTGCGTCTGCCTCAGAAGGACTCCCCAAACCAGGA
 Q  S  E  A  H  G  L  P  L  R  L  P  Q  K  D  S  P  N  Q  D

       490       500       510       520       530       540
TGCAACATCCTGGGGACCTGTGCGCTTCCTGCCCATGCCAGGCCTGCTCCACGAGCCCCA
 A  T  S  W  G  P  V  R  F  L  P  M  P  G  L  L  H  E  P  Q
```

Fig. 4A

```
       550        560        570        580        590        600
AGACCAAGCAGGATTCCTGCCCCCAGAGCCCCCAGATGTGGGCTCCTCTGACCCCCTGAG
 D  Q  A  G  F  L  P  P  E  P  P  D  V  G  S  S  D  P  L  S

       610        620        630        640        650        660
CATGGTAGAGCCTTTACAGGGCCGAAGCCCCAGCTATGCGTCCTGACTCTTCCTGAATC
 M  V  E  P  L  Q  G  R  S  P  S  Y  A  S  *
```

Fig. 4B

Filename: human FGF-21 cDNA in pGEM-T
Sequence Size: 643
Sequence Position: 1 - 643
Translation Position: 9 - 638;

```
        10         20         30         40         50         60
agccattgatggactcggacgagaccgggttcgagcactcaggactgtgggtttctgtgc  SEQ ID NO:3
       M  D  S  D  E  T  G  F  E  H  S  G  L  W  V  S  V  L    SEQ ID NO:4

        70         80         90        100        110        120
tggctggtcttctgctgggagcctgccaggcacaccccatccctgactccagtcctctcc
  A  G  L  L  L  G  A  C  Q  A  H  P  I  P  D  S  S  P  L  L

       130        140        150        160        170        180
tgcaattcgggggccaagtccggcagcggtacctctacacagatgatgcccagcagacag
  Q  F  G  G  Q  V  R  Q  R  Y  L  Y  T  D  D  A  Q  Q  T  E

       190        200        210        220        230        240
aagcccacctggagatcagggaggatgggacggtgggggcgctgctgaccagagccccg
  A  H  L  E  I  R  E  D  G  T  V  G  G  A  A  D  Q  S  P  E

       250        260        270        280        290        300
aaagtctcctgcagctgaaagccttgaagccgggagttattcaaatcttgggagtcaaga
  S  L  L  Q  L  K  A  L  K  P  G  V  I  Q  I  L  G  V  K  T

       310        320        330        340        350        360
catccaggttcctgtgccagcggccagatggggccctgtatggatcgctccactttgacc
  S  R  F  L  C  Q  R  P  D  G  A  L  Y  G  S  L  H  F  D  P

       370        380        390        400        410        420
ctgaggcctgcagcttccgggagctgcttcttgaggacggatacaatgtttaccagtccg
  E  A  C  S  F  R  E  L  L  L  E  D  G  Y  N  V  Y  Q  S  E

       430        440        450        460        470        480
aagcccacggcctcccgctgcacctgccagggaacaagtccccacaccgggaccctgcac
  A  H  G  L  P  L  H  L  P  G  N  K  S  P  H  R  D  P  A  P

       490        500        510        520        530        540
cccgaggaccagctcgcttcctgccactaccaggcctgccccccgcactcccggagccac
  R  G  P  A  R  F  L  P  L  P  G  L  P  P  A  L  P  E  P  P
```

Fig. 5A

```
      550       560       570       580       590       600
ccggaatcctggccccccagccccccgatgtgggctcctcggaccctctgagcatggtgg
  G  I  L  A  P  Q  P  P  D  V  G  S  S  D  P  L  S  M  V  G

      610       620       630       640       650
gaccttcccagggccgaagccccagctacgcttcctgaagcca
  P  S  Q  G  R  S  P  S  Y  A  S  *
```

Fig. 5B

```
human FGF-21  MDSDETGFEHSGLWVS-VLAGLLLGACQAHPIPDSSPLLQFGGQVRQRYLYTDDAQQTEA  59
              *          ****   **  ***  ** ************************ * ***
mouse FGF-21  MEWMRSRVGTLGLWVRLLLAVFLLGVYQAYPIPDSSPLLQFGGQVRQRYLYTDDDQDTEA  60

              HLEIREDGTVGGAADQSPESLLQLKALKPGVIQILGVKTSRFLCQRPDGALYGSLHFDPE  119
              ********** ***  ****** *************** ****** ******** *****
              HLEIREDGTVVGAAHRSPESLLELKALKPGVIQILGVKASRFLCQQPDGALYGSPHFDPE  120

              ACSFRELLLEDGYNVYQSEAHGLPLHLPGNKSPHRDPAPRGPARFLPLPGLPPALPEPPG  179
              ************************** **   ** *   ** **** ***        *
              ACSFRELLLEDGYNVYQSEAHGLPLRLPQKDSPNQDATSWGPVRFLPMPGLLHEPQDQAG  180

              ILAPQPPDVGSSDPLSMVGPSQGRSPSYAS   209
               * * ************* * *********
              FLPPEPPDVGSSDPLSMVEPLQGRSPSYAS   210
```

Fig. 6

Codon usage for yeast (highly expressed) genes

AmAcid	Codon	Number	/1000	Fraction
Gly	GGG	33.00	0.86	0.01
Gly	GGA	70.00	1.82	0.02
Gly	GGT	2672.00	69.62	0.91
Gly	GGC	171.00	4.46	0.06
Glu	GAG	277.00	7.22	0.10
Glu	GAA	2442.00	63.63	0.90
Asp	GAT	1100.00	28.66	0.48
Asp	GAC	1211.00	31.55	0.52
Val	GTG	117.00	3.05	0.04
Val	GTA	75.00	1.95	0.03
Val	GTT	1548.00	40.33	0.56
Val	GTC	1026.00	26.73	0.37
Ala	GCG	36.00	0.94	0.01
Ala	GCA	203.00	5.29	0.06
Ala	GCT	2221.00	57.87	0.65
Ala	GCC	969.00	25.25	0.28
Arg	AGG	20.00	0.52	0.01
Arg	AGA	1336.00	34.81	0.83
Ser	AGT	116.00	3.02	0.05
Ser	AGC	94.00	2.45	0.04
Lys	AAG	2365.00	61.62	0.78
Lys	AAA	651.00	16.96	0.22
Asn	AAT	347.00	9.04	0.22
Asn	AAC	1259.00	32.80	0.78
Met	ATG	766.00	19.96	1.00
Ile	ATA	43.00	1.12	0.02
Ile	ATT	1223.00	31.87	0.52
Ile	ATC	1070.00	27.88	0.46
Thr	ACG	28.00	0.73	0.01

Fig. 7A

Thr	ACA	126.00	3.28	0.06
Thr	ACT	1129.00	29.42	0.50
Thr	ACC	962.00	25.07	0.43
Trp	TGG	325.00	8.47	1.00
End	TGA	10.00	0.26	0.09
Cys	TGT	254.00	6.62	0.89
Cys	TGC	33.00	0.86	0.11
End	TAG	11.00	0.29	0.10
End	TAA	85.00	2.21	0.80
Tyr	TAT	219.00	5.71	0.19
Tyr	TAC	913.00	23.79	0.81
Leu	TTG	2202.00	57.38	0.69
Leu	TTA	576.00	15.01	0.18
Phe	TTT	432.00	11.26	0.27
Phe	TTC	1145.00	29.83	0.73
Ser	TCG	26.00	0.68	0.01
Ser	TCA	149.00	3.88	0.06
Ser	TCT	1279.00	33.33	0.52
Ser	TCC	818.00	21.31	0.33
Arg	CGG	0.00	0.00	0.00
Arg	CGA	1.00	0.03	0.00
Arg	CGT	249.00	6.49	0.15
Arg	CGC	5.00	0.13	0.00
Gln	CAG	62.00	1.62	0.05
Gln	CAA	1225.00	31.92	0.95
His	CAT	236.00	6.15	0.35
His	CAC	433.00	11.28	0.65
Leu	CTG	52.00	1.35	0.02
Leu	CTA	236.00	6.15	0.07
Leu	CTT	90.00	2.35	0.03
Leu	CTC	14.00	0.36	0.00
Pro	CCG	10.00	0.26	0.01
Pro	CCA	1271.00	33.12	0.80
Pro	CCT	279.00	7.27	0.18
Pro	CCC	33.00	0.86	0.02

Fig. 7B

Codon usage for Drosophila (highly expressed) genes

AmAcid	Codon	Number	/1000	Fraction
Gly	GGG	6.00	0.28	0.00
Gly	GGA	380.00	18.04	0.22
Gly	GGT	575.00	27.29	0.34
Gly	GGC	746.00	35.41	0.44
Glu	GAG	1217.00	57.77	0.91
Glu	GAA	115.00	5.46	0.09
Asp	GAT	503.00	23.88	0.43
Asp	GAC	654.00	31.04	0.57
Val	GTG	719.00	34.13	0.45
Val	GTA	29.00	1.38	0.02
Val	GTT	226.00	10.73	0.14
Val	GTC	608.00	28.86	0.38
Ala	GCG	94.00	4.46	0.05
Ala	GCA	80.00	3.80	0.04
Ala	GCT	446.00	21.17	0.24
Ala	GCC	1277.00	60.61	0.67
Arg	AGG	48.00	2.28	0.06
Arg	AGA	12.00	0.57	0.01
Ser	AGT	16.00	0.76	0.01
Ser	AGC	267.00	12.67	0.23
Lys	AAG	1360.00	64.55	0.93
Lys	AAA	108.00	5.13	0.07
Asn	AAT	127.00	6.03	0.13
Asn	AAC	878.00	41.67	0.87
Met	ATG	387.00	18.37	1.00
Ile	ATA	4.00	0.19	0.00
Ile	ATT	390.00	18.51	0.29
Ile	ATC	969.00	45.99	0.71

Fig. 8A

Thr	ACG	114.00	5.41	0.08
Thr	ACA	34.00	1.61	0.02
Thr	ACT	164.00	7.78	0.11
Thr	ACC	1127.00	53.49	0.78
Trp	TGG	243.00	11.53	1.00
End	TGA	1.00	0.05	0.01
Cys	TGT	20.00	0.95	0.08
Cys	TGC	220.00	10.44	0.92
End	TAG	12.00	0.57	0.17
End	TAA	58.00	2.75	0.82
Tyr	TAT	113.00	5.36	0.16
Tyr	TAC	574.00	27.25	0.84
Leu	TTG	210.00	9.97	0.12
Leu	TTA	9.00	0.43	0.01
Phe	TTT	62.00	2.94	0.09
Phe	TTC	635.00	30.14	0.91
Ser	TCG	195.00	9.26	0.17
Ser	TCA	29.00	1.38	0.02
Ser	TCT	103.00	4.89	0.09
Ser	TCC	558.00	26.49	0.48
Arg	CGG	7.00	0.33	0.01
Arg	CGA	25.00	1.19	0.03
Arg	CGT	281.00	13.34	0.34
Arg	CGC	465.00	22.07	0.55
Gln	CAG	703.00	33.37	0.91
Gln	CAA	66.00	3.13	0.09
His	CAT	88.00	4.18	0.22
His	CAC	312.00	14.81	0.78
Leu	CTG	1182.00	56.10	0.69
Leu	CTA	21.00	1.00	0.01
Leu	CTT	55.00	2.61	0.03
Leu	CTC	224.00	10.63	0.13
Pro	CCG	84.00	3.99	0.09
Pro	CCA	135.00	6.41	0.15
Pro	CCT	72.00	3.42	0.08
Pro	CCC	626.00	29.71	0.68

Fig. 8B

Codon usage for enteric bacterial (highly expressed) genes 7/19/83

AmAcid	Codon	Number	/1000	Fraction
Gly	GGG	13.00	1.89	0.02
Gly	GGA	3.00	0.44	0.00
Gly	GGU	365.00	52.99	0.59
Gly	GGC	238.00	34.55	0.38
Glu	GAG	108.00	15.68	0.22
Glu	GAA	394.00	57.20	0.78
Asp	GAU	149.00	21.63	0.33
Asp	GAC	298.00	43.26	0.67
Val	GUG	93.00	13.50	0.16
Val	GUA	146.00	21.20	0.26
Val	GUU	289.00	41.96	0.51
Val	GUC	38.00	5.52	0.07
Ala	GCG	161.00	23.37	0.26
Ala	GCA	173.00	25.12	0.28
Ala	GCU	212.00	30.78	0.35
Ala	GCC	62.00	9.00	0.10
Arg	AGG	1.00	0.15	0.00
Arg	AGA	0.00	0.00	0.00
Ser	AGU	9.00	1.31	0.03
Ser	AGC	71.00	10.31	0.20
Lys	AAG	111.00	16.11	0.26
Lys	AAA	320.00	46.46	0.74
Asn	AAU	19.00	2.76	0.06
Asn	AAC	274.00	39.78	0.94
Met	AUG	170.00	24.68	1.00
Ile	AUA	1.00	0.15	0.00
Ile	AUU	70.00	10.16	0.17
Ile	AUC	345.00	50.09	0.83
Thr	ACG	25.00	3.63	0.07
Thr	ACA	14.00	2.03	0.04
Thr	ACU	130.00	18.87	0.35

Fig. 9A

AmAcid	Codon	Number	/1000	Fraction
Thr	ACC	206.00	29.91	0.55
Trp	UGG	55.00	7.98	1.00
End	UGA	0.00	0.00	0.00
Cys	UGU	22.00	3.19	0.49
Cys	UGC	23.00	3.34	0.51
End	UAG	0.00	0.00	0.00
End	UAA	0.00	0.00	0.00
Tyr	UAU	51.00	7.40	0.25
Tyr	UAC	157.00	22.79	0.75
Leu	UUG	18.00	2.61	0.03
Leu	UUA	12.00	1.74	0.02
Phe	UUU	51.00	7.40	0.24
Phe	UUC	166.00	24.10	0.76
Ser	UCG	14.00	2.03	0.04
Ser	UCA	7.00	1.02	0.02
Ser	UCU	120.00	17.42	0.34
Ser	UCC	131.00	19.02	0.37
Arg	CGG	1.00	0.15	0.00
Arg	CGA	2.00	0.29	0.01
Arg	CGU	290.00	42.10	0.74
Arg	CGC	96.00	13.94	0.25
Gln	CAG	233.00	33.83	0.86
Gln	CAA	37.00	5.37	0.14
His	CAU	18.00	2.61	0.17
His	CAC	85.00	12.34	0.83
Leu	CUG	480.00	69.69	0.83
Leu	CUA	2.00	0.29	0.00
Leu	CUU	25.00	3.63	0.04
Leu	CUC	38.00	5.52	0.07
Pro	CCG	190.00	27.58	0.77
Pro	CCA	36.00	5.23	0.15
Pro	CCU	19.00	2.76	0.08
Pro	CCC	1.00	0.15	0.00

Fig. 9B

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
25 May 2001 (25.05.2001)

PCT

(10) International Publication Number
WO 01/36640 A3

(51) International Patent Classification[7]: C12N 15/12, C07K 14/50, C12N 5/10, C07K 16/22, A61K 38/18

(21) International Application Number: PCT/US00/31745

(22) International Filing Date:
16 November 2000 (16.11.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/166,540 18 November 1999 (18.11.1999) US
60/203,633 11 May 2000 (11.05.2000) US

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(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

(88) Date of publication of the international search report:
11 October 2001

[Continued on next page]

(54) Title: HUMAN FGF-21 GENE AND GENE EXPRESSION PRODUCTS

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human FGF-21    MDSDETGFEHSGLWVSVLAGLLLGACQAHPIPDSSPLLQFG-GQV-RQRYLYTD  52
                         ** * * * *    * **  * *  * ***
mouse FGF-15    MARKWNGRAVARALVLATLWLAVS-GRPLAQ-QSQSVSDEDPLFLYGWGKITRLQYLYSA  58

                DAQQTEAHLEIREDGTVGGAADQSPESLLQLKALKPGVIQILGVKTSRFLCQRPDGALYG  112
                      * ** ** *    **   **  *    * * *  * **  ** **
                GPYVSNCFLRIRSDGSVDCEEDQNERNLLEFRAVALKTIAIKDVSSVRYLCMSADGKIYG  118

                SLHFDPEACSFRELLLEDGYNVYQSEAHGLPLHLPGNKSPHRDPAPRGPARFLPLPGLPP  172
                    * * ***    *** * *  * *      * *       * * *
                LIRYSEEDCTFREEMDCLGYNQYRSMKHHLHIIFIQAK-PREQLQDQKPSNFIPVFHRSF  177

                ALPEPPGILAPQ--PPDVGSSDPLSMVGPSQG--RSPSYAS
                             *   * **  **       ***
                FETGDQLRSKMFSLPLESDSMDPFRMVEDVDHLVKSPSFQK
```

(57) Abstract: This invention relates to human fibroblast growth factor (hFGF-21), and to variants thereof and to polynucleotides encoding FGF-21. The invention also relates to diagnostic and therapeutic agents related to the polynucleotides and proteins, including probes and antibodies, and to methods of treating liver disease such as cirrhosis and cancer, methods of treating conditions related to thymic function, and methods of treating conditions of the testis. The invention also relates to mouse fibroblast growth factor (mFGF-21), and to variants thereof and polynucleotides encoding mFGF-21.

WO 01/36640 A3

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INTERNATIONAL SEARCH REPORT

In ational Application No
PCT/US 00/31745

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/12 C07K14/50 C12N5/10 C07K16/22 A61K38/18

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, WPI Data, PAJ, STRAND

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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X	DATABASE EMBL SEQUENCE LIBRARY 'Online! 27 August 1997 (1997-08-27) KODA, Y., ET AL. : "changing transcription start sites in H-type alpha (1,2) fucosyltransferase gene (FUT1) during differentiation of the human erythroid lineage" XP002165898 accession no. AB006136 --- -/--	1-4,6, 12,14-18

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Date of the actual completion of the international search	Date of mailing of the international search report
24 April 2001	10/05/2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Holtorf, S

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INTERNATIONAL SEARCH REPORT

Ir. ational Application No
PCT/US 00/31745

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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